ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE ON AGING
FISCAL YEAR 1979

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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NIA ANNUAL REPORT

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NIA ANNUAL REPORT
OCTOBER 1, 1978 THROUGH SEPTEMBER 30, 1979
OFFICE OF THE DIRECTOR

Fiscal Year 1979 was marked by a broadening and deepening of NIA research programs and interests, ranging from a proposed reorganization of the extramural program to plans for two new types of grant mechanisms and implementation of a third. NIA staff has also continued to encourage research proposals through targeted program announcements and conferences. An indication of the success of these efforts is the fivefold increase in grant applications over the past 4 years. Among the most notable developments are the following:

Reorganization of the Extramural Program

The NIA reorganized its Extramural and Collaborative Research Program and proposed the following three organizational branches: Biomedical Research and Clinical Medicine (BRCM); Behavioral and Social Sciences (BSS); and Planning and Extramural Affairs (PEA). Donald G. Murphy, Ph.D., was appointed Acting Associate Director, BRCM; Matilda White Riley, Ph.D., was named Associate Director, BSS; and Don C. Gibson, D.V.M., was appointed Associate Director, PEA.

Program Announcements

NIA program announcements published in the NIH Guide to Grants and Contracts during FY 1979 covered a wide range of interests. Within BRCM, the Basic Aging program issued two announcements on the genetic basis of aging; the Molecular and Biochemical Aging program announced an expanded program in immunology; and the Biophysiology and Pathobiology program solicited applications on the function of the aging musculoskeletal system. The Behavioral and Social Sciences program announced a request for applications for studies on the psychosocial aspects of aging within minority groups. The NIA also cooperated with other Institutes in cosponsoring announcements on overnutrition and obesity, Clinical Nutrition Research Units, and behavioral studies related to diabetes mellitus.

Applications received for funding under the Geriatric Medicine Academic Award, which was initiated in FY 1978, resulted in eleven grants awarded in FY 1979. The NIA also announced its plans to fund training via two NIH mechanisms--the Fogarty Senior Internanational Fellowship and National Research Service Award for Senior Fellows. Both awards will enable experienced investigators to broaden their knowledge of aging research.

Behavioral and Social Sciences Research

The NIA research program in the behavioral and social sciences has grown from about 14 percent of the NIA's research program when the Institute was established in 1974 to 25 percent in 1979. This growth reflects heightened awareness of the importance of the psychological and social aspects of aging, especially as they relate to physical and mental health.

The Behavioral Science program concentrates on behavioral changes and adaptation to aging. These include studies of: memory, learning, and cognitive changes in the aged; problem-solving behavior of middle-aged and elderly adults; how the senses of taste and smell change with age, and how these changes contribute to inadequate nutrition in the aged; how older people perceive and deal with stress; and behavioral characterizations of the menopausal hot flash.

The Social Science research program explores the conditions of older people in the context of various social institutions and cultural influences. The program includes studies of: family structure and lifestyle; the aging of ethnic minorities; the psychological and physiological impact of bereavement; demography and migration of the aged; attitudes toward the elderly; and work and retirement.

Future efforts in the Behavioral and Social Sciences research program will be directed toward developing studies on: the rural elderly; service delivery to the elderly; stress and its relation to disease; families and social support systems of the aged; minority aging; and the influence of the "middle years" upon the later years of life.

Consensus Activities

As part of an NIH-wide effort to communicate the best informed opinion with regard to the safety and efficacy of new procedures or diagnostic techniques, the NIA took part in several consensus development activities during FY 1979. Throughout the year, NIA staff and a task force of experts continued work on a statement to aid practicing physicians in detecting, diagnosing, and evaluating the treatable brain disorders which often mimic irreversible brain disease in elderly patients. Senility Reconsidered: Treatment Possibilities Impairment in the Elderly, which is now in the final stages of review, will be the end product of a consensus development meeting held in the summer of 1978. This paper will be widely disseminated to physicians, other health providers, and those who work with the elderly.

As one of several Federal agencies which constitute the Interagency Committee on New Therapies for Pain and Discomfort, the NIA cosponsored a conference on Pain, Discomfort, and Humanitarian Care on February 15 and 16, 1979. As part of that meeting, a consensus development panel on the management of pain and discomfort in the terminally ill looked at such issues as care of the aged, death and dying, and the hospice approach to terminal illness.

In September 1979, the NIA brought biomedical researchers together with practicing physicians, representatives of women's groups, and the general public to weigh the risks and benefits of estrogen use and various types of estrogen therapy in managing menopausal and postmenopausal symptoms and conditions. Recent actions by the Food and Drug Administration as well as an increasing number of articles in the popular press have made this subject one of great interest and concern for women of all ages.

Workshops and Conferences

The NIA held or supported a number of workshops and conferences to review the state of knowledge in each area, draw attention to research needs, and stimulate investigators to submit applications for research grants designed to increase understanding of the subjects. Among the meetings that took place in FY 1979 are:

On January 18 and 19, 1979, the NIA sponsored a forum on Thymic Hormones and Aging, at which four eminent researchers in the field of immunology presented papers about several different kinds of thymic hormones, including serum thymic factor, thymosin, and thymopoietin. Consultants and speakers then discussed the production, testing, and distribution of thymic hormones for use in aging research.

A conference on the Immunological Aspects of Aging, held May 7 and 8, 1979, brought together investigators active in such areas as cell-mediated immunity, T- and B-cell interaction, nutrition and immunity, immunoendocrinology, autoimmunity, clinical expressions of immunodeficiencies, and other diseases which occur in the elderly.

On May 8 and 9, 1979, the NIA and NIDR cosponsored a conference on Oral Motor Behaviors: Impact on Oral Conditions and Dental Treatment. Among the issues discussed at this meeting were special oral problems of the elderly, implementation of prostheses, and recommendations for research on dental problems in the elderly.

A Workshop on Nutrition, Behavior, and the Life Cycle, jointly sponsored by the NIA and the National Institute of Child Health and Human Development on June 18-20, 1979, brought together 25 participants from diverse fields to explore the gaps in current knowledge of nutrition and behavior. The emphasis was on multidisciplinary approaches to studies of food selection and food intake. Changes in food preference can occur throughout the life span and can result in undernourishment in old age. A better understanding of the mechanisms involved in changing patterns of food selection and food intake will be useful in studying lifelong obesity as well as obesity occurring with advancing age.

The Conference on Demographic and Health Information for Aging Research: Resources and Needs was sponsored by NIA on June 25-27, 1979 to determine the availability of statistical data on the health, socioeconomic, and other needs of an aging U.S. population. Government and nongovernment researchers, policymakers, and other users of Federal data on the elderly met to discuss existing data and to suggest additional information needed for aging research, to make data collection agencies aware of the research uses being made of their data, and to suggest ways to coordinate and increase the value of that information without jeopardizing the basic reasons for collecting it.

From the earliest days of childhood through the last days of life, auditory communications play a major role in learning, socialization, and day-to-day interactions. On August 9 and 10, 1979, the NIA joined with the National Institute of Neurological and Communicative Disorders and Stroke and the Division of Research Resources in sponsoring a seminar on Hearing Problems of the Aged. This seminar was designed as a forum for discussion of the scientific, clinical, and technological issues which require the special attention of investigators and clinicians. Hearing loss represents a significant problem for the elderly population, yet there is much that we do not know about the prevalence of hearing loss, differential diagnosis in patients with hearing loss, and how modern technology might be applied to alleviate their problems.

Geriatric Medicine

The NIA's interest in the teaching of geriatric medicine continued during FY 1979, boosted by several events. The Institute of Medicine of the National Academy of Sciences submitted its report on Aging and Medical Education, which was prepared under contract to the NIA. The report found that although there is an extensive body of knowledge on aging and care of the aged that is relevant to medical education, gerontology and geriatrics are not incorporated adequately within the majority of medical schools and graduate medical education programs. The study committee urged the systematic inclusion of this knowledge at all levels of physician training, but strongly discouraged the development of a formal practice specialty in geriatrics. Rather, the report favored the recognition of gerontology and geriatrics as academic disciplines within the appropriate medical specialties.

Later in FY 1979, the Institute of Medicine provided NIA with a comprehensive survey of undergraduate courses in geriatrics and gerontology in American medical schools. This followup report was undertaken at NIA's request, and should prove to be a valuable resource for students interested in the care of the elderly.

The NIA's nomination of Sir Ferguson Anderson, one of the world's foremost geriatricians, to be a Fogarty International Scholar was accepted. He plans to begin his year with the NIA in October 1979, participating in a variety of activities designed to speed the transmission of knowledge regarding geriatric medicine to American medical school students and faculty, as well as policymakers and scientists in the field of aging.

In late 1978, the Fogarty International Center's Senior International Fellowship Program (also known as the Magnuson award) was given an additional $200,000.00 to be used in coordination with the NIA to provide fellowships for midlevel and senior scientists to share their expertise and gain career-enhancing experience in some of the most advanced geriatric centers of the world. Also in FY 1979, the Bureau of Health Manpower of the Health Resources Administration was given up to $2 million by Congress for curriculum development in geriatrics. With the advice and counsel of the NIA, specific guidelines were developed for the use of these funds through the mechanism of curriculum development grants.

Geriatric Dentistry

The NIA's interest in geriatrics extends beyond medicine to dentistry and other specialized areas of clinical research. In FY 1979, all three programs of the Institute became involved in geriatric dentistry. The Intramural Program at the Gerontology Research Center in Baltimore, Maryland, added a dental evaluation component to the already extensive physical and mental examination protocol used in the Baltimore Longitudinal Study of Aging. The dental protocol includes clinical evaluation of gum diseases, cavities, changes in the senses of taste and smell, oral hygiene, salivary and related oral gland function, and alterations in denture-retaining ridges. Biochemical tests are also performed on saliva samples.

The Extramural Program is coordinating with the National Institute of Dental Research to identify areas of overlapping interest and high priority with the eventual goal of funding research on the many aspects of geriatric dentistry. Topics of interest to both Institutes include: root cavities, gum disease, oral tissue changes, wound healing, immune capacity, elderly cleft palate, removable dental appliances, loss of teeth, oral bone loss, nutrition, oral cancer, denture sores, salivary function, therapy compliance, oral hygiene, and taste and smell changes which accompany aging.

To encourage the development of a quality curriculum in geriatric dentistry that will attract outstanding students to aging research and contribute to the improved dental care of the aged, the NIA in cooperation with the NIDR has announced the availability of NIA Geriatric Dentistry Academic Awards. The awards provide support to individual faculty members for their educational development and for the implementation of curricula in geriatric dentistry.

The Epidemiology, Demography, and Biometry Program (EDBP) of the NIA has taken steps to initiate the compilation of dental information from already existing surveys. With guidance and funding from the NIA, data from the Health and Nutrition Examination Survey (HANES) of the National Center for Health Statistics is being reexamined closely to collect important information on the older population. Once the followup data have been collected, the EDBP hopes to undertake a special examination of the relationship between dental problems and nutrition in the elderly.

Collaboration

In addition to the collaborative efforts with NIH Institutes and other Federal agencies described above, the NIA has interacted with the National Heart, Lung, and Blood Institute to study hypertension; with the National Institute of Mental Health and the National Institute of Neurological and Communicative Disorders and Stroke on senile dementia and other types of mental impairment in old age; with the National Aeronautics and Space Administration to explore ways to adapt space-age technology to the development of prosthetic devices to the elderly; and with the Administration on Aging in a number of areas of mutual interest. The Institute has also maintained active liaison with the many private organizations which represent older Americans, including the Gerontological Society, the American Geriatrics Society, the National Council of Senior Citizens, the American Association of Retired Persons/National Retired Teachers

Association, and the National Council on the Aging. We hope to continue such cooperative efforts, for they not only maximize the resources of a small Institute, they also bring a variety of views and backgrounds to bear on the complex process of aging.

INFORMATION OFFICE

The NIA Information Office continued during Fiscal Year 1979 to increase public awareness of Institute programs and interests. Special attention was given to developing ways of reaching specific segments of the public with targeted messages on topics likely to directly affect each group. The various methods used for distributing this information included publishing brochures and articles; conducting public information campaigns and press conferences; preparing summaries of selected NIA conferences; exhibiting at national conventions and meetings; and responding to letter and telephone inquiries from the public, press, physicians, scientists, students, health care professionals, and Congress.

The following is a list of brochures published during Fiscal Year 1979:

- Q & A: Alzheimer's Disease -- a description of the state of knowledge regarding the causes, symptoms, treatment, and current research on Alzheimer's disease, senile dementia, and related disorders.

- Index of NIA Grants and Contracts -- a list of NIA-funded research and training grants and contracts. (technical assistance)

- Protection of Elderly Research Subjects -- the summary of a conference sponsored by the NIA on July 18-19, 1977. Conference topics concerned the importance of conducting biomedical research on aging on human subjects and what precautions should be taken to protect these subjects.

- Development of the Rodent as a Model System of Aging, Book II -- a technical publication intended to present an accurate guide on rodents, the standard research animal used to study the processes of aging. The book details behavioral characteristics, age specific pathology, disease susceptibility, and breeding habits. (technical assistance)

- International Perspectives on Aging -- testimony presented before the U.S. Senate Special Committee on Aging by Robert N. Butler, M.D., Director of the NIA. This discussion reflects some of the thoughts and goals of the international research community regarding research on aging.

- Observations in Geriatric Medicine -- this publication is a description of the scope of geriatrics by an expert in the field, as well as a device to recruit new physicians to geriatric medicine from the ranks of medical students. In addition, the material is a valuable supplement to general medical texts, which often contain little information on normal aging or the diseases which may accompany old age.

- Aging and Medical Education -- the report of a study on the incorporation of material on geriatrics and gerontology in medical education, conducted by the Institute of Medicine of the National Academy of Sciences. (the IO facilitated printing and distribution)

- Recent Developments in Clinical and Research Geriatric Medicine: the NIA Role -- a review of recent developments in geriatric medicine throughout the U.S., including descriptions of current programs and training opportunities open to physicians, medical students, and other health care professionals.

- Cells and Aging and Does Intelligence Decline with Age? -- summaries of presentations given at the November 1977 State of the Art Seminar on Aging Research in San Francisco, California.

- The original 9 Science Writer Seminar brochures were revised and reprinted.

In cooperation with the Director, the Information Office prepared the following articles for publication in various journals and books oriented toward audiences ranging from professional to lay:

- "Compassion and Relief from Pain" and "Life Review: An Unrecognized Bonanza" -- Public Interest Columns for International Journal of Aging and Human Development

- Forward to American Personnel and Guidance Association Training Material

- "Pain in the Elderly" for Journal of the American Medical Association

- Forward for Current Practices in Gerontological Nursing

- "Old Age" for Encyclopedia Americana

- "Geriatric Medicine: Training, Teaching, Research, and Service" for Sir Ferguson Anderson

- "Overview of the Pharmacology of Aging" for Excerpta Medica

- "Overview on Aging" for Aging: The Process and the People

- "Aging and Mental Health" chapter for Clinical Geriatrics

- "What to do for the Old Folks" for Ann Landers

- "Growing Old Gracefully" for Encyclopedia Britannica

- "Thoughts on Geriatric Medicine" for American Health Care Association Journal

- Forward to Aging in Non-Human Primates

- "Drugs and the Elderly" for the United Methodist Church

- Statement on Thanatologic Aspects of Aging

- "We are Asking the Wrong Questions: Thoughts on the Economics of Aging" for National Journal

- Statement on the Older Woman for Vogue

- Forward to Old Age in the New Land

- "Cancer and Aging: Research Perspectives and Potentials" for CA, a publication of the American Cancer Society

- "Care of the Elderly" for Resident and Staff Physician

- "Care of the Aged: Perspectives on Pain and Discomfort" for chapter in conference proccedings

- "Senile Dementia of the Alzheimer's Type: Research Leads and Research Needs" for Forum on Medicine

- "Hearing and Age: Research Challenges and the NIA" for Annals of Otology, Rhinology and Laryngology

- Preface for Summary on Workshop on Sleep and Aging for Sleep Journal

- "Skin Aging," "Older Woman," "Drugs and Aging," and "Treatable Dementia" -- Search for Health Articles (to local newspapers throughout the country)

- Letter to the Editor of Newsweek

- Preface to Sexuality in the Later Years

- "Perspectives on Mental Health and Mental Illness in Old Age" for Generations

- "The Need for Quality Hospice Care" for QRB and Death Education

- "Self Care, Self Help and the Elderly" for International Journal of Aging and Human Development

- Statement on Chair of Geriatrics at Cornell

- Editorial for Alzheimer's Disease Society Newsletter

- "Aging Healthfully in Mind and Spirit" for Woman Doctors' Health Guide and Medical Encyclopedia

- Letter on asbestosis for National Cancer Institute campaign

- "Helping the Elderly" for Journal of Home Economics

- "Health Promotion and Disease Prevention in Old Age" for Julius Richmond in Aging

- "Prospects for Middle-Aged Women" for Women in Midlife--Security and Fulfillment, Part I (Select Committee on Aging, U.S. House of Representatives)
- "The Normal Psychology of the Aging Process" (edited book review)
- Article by Drs. Gaarder and Butler for Psychosomatics Review (editing)
- "Recent Developments in Clinical Gerontology and Geriatric Research in America" for Geriatrics Review
- "The Relationship of Geriatrics to Internal Medicine" for Annals of Internal Medicine
- "Ageism" for Journal of Social Issues

The Information Office also prepared the following NIH Record articles:

- Mayor Schaefer Visits GRC
- Dr. Butler Receives APA Founders Award
- Dr. Pickett Receives Brown University Award
- Dr. Milo Leavitt Named NIA Director's Assistant
- Dr. Ringler Appointed Deputy Director
- Conference on The Older Woman: Continuities and Discontinuities
- Dr. Castel Appointed to NIA
- Dr. Duncan Retires
- Dr. Rapoport Joins NIA
- Dr. Eichhorn Named Maryland Chemist of the Year
- Conference on Thymic Hormones and Aging
- Conference on Nutrition, Behavior, and the Life Cycle
- Conference on Epidemiologic Aspects of Aging
- Conference on Immunological Aspects of Aging
- Conference on Pain, Discomfort, and Humanitarian Care
- Dan Rogers: Handicapped Marylander of the Year
- Board of Scientific Counselors

Other projects completed during the year included the preparation of press announcements, speeches, NIA sections of annual NIH publications, and press activities at selected NIA conferences.

Press Announcements:

- Conference on Nutritional Needs and Health of the Aged Adult
- Consensus Meeting on Treatable Brain Disease in the Elderly
- Backgrounder on Treatable Brain Diseases
- Backgrounder on Interagency Committee on New Therapies for Pain and Discomfort
- Mr. Chicchirichi, NIH Director's Award
- Dr. Ringler appointed Deputy Director
- Consensus Meeting on Pain, Discomfort, and Humanitarian Care
- Publication Announcement for Our Future Selves and four Panel Reports
- Aging and Medical Education
- Publication Announcement for To Understand the Aging Process
- Publication Announcement for Changes
- Research by Dr. Masoro
- A Winter Hazard for the Elderly
- Clinical Nutrition Research Units (joint release)
- Workshop on Immunological Aspects of Aging
- Conference on Demographic and Health Information for Aging Research: Resources and Needs
- Conference on The Older Woman: Continuities and Discontinuities
- Backgrounder on The Older Woman
- Did Methusaleh Lie About His Age? (The International Workshop of Longevous Population of Vilcabamba, Ecuador)
- Publication Announcement for NIA Publication List
- Publication Announcement for Special Report 1979
- Geriatric Medicine Academic Award
- Nutrition Update

Speeches:

- Hearing and Age
- Speech to National Conference on County Resources Development for Aging Citizens (original 1-77; revised for publication 4-79)
- Pharmacological Interventions of the Aging Process
- Aging in America for National Bureau of Standards (abstract)
- The Future of Aging Research in the United States -- a special lecture at the American Psychiatric Association meeting
- Why Survive: 2078
- Remarks on Geriatric Medicine (edit for publication by the National Academy of Sciences)
- NIA: Practical Goals in Aging
- The Graying of Nations: Creative Responses (for Age Concern)
- Opening Remarks for the Conference on The Older Woman (edit for publication)
- The Need for Quality Hospice Care
- Man and Aging: Perspectives on Pain and Discomfort
- Remarks on Retirement
- Remarks at WHO-sponsored Symposium on Society, Stress, and Disease: Aging and Old Age (edit for publication)
- Symposium on the Care of the Elderly at the University of Massachusetts (edit)
- Opening remarks for Dr. Ringler at Conference on Clinical Aspects of Alzheimer's Disease and Senile Dementia

Annual Reports:

- Annual Report (1978)
- NIH Almanac (1978)
- Scientific Directory/Bibliography 1979 (published twice yearly)
- Highlights in the History and Organization of the NIH

- Special Report on Aging 1979 (Senate)

- Developments in Aging 1979 (House of Representatives)

In addition to the activities listed above, the Information Office is involved in a number of special projects. These range from creating a hall exhibit to welcome visitors to NIA to cosponsoring a conference on Mass Media and Aging at the Columbia University School of Journalism.

Special projects now in process include:

- A videotape describing NIA for visitors and new employees

- Pretesting NIA publications and audiovisual products to evaluate their effectiveness

- Public service announcements for television and radio on myths about aging and on accidental hypothermia

- Planning of a meeting with self-help groups for the families of those with Alzheimer's disease

- Translation of classic cases in aging

Miscellaneous:

An important function of the Information Office has been to disseminate information materials as widely as possible to target audiences. Thus, the rapidly increasing number of requests for NIA publications made it necessary for the Institute to begin, in May 1979, contracting with a private company to handle our addressing and mailing tasks. Compared with last year, we have been able to increase by about 25,000 our distribution of pamphlets, speeches, and booklets in response to individual requests, mailing keys, and meetings of organizations interested in the aged. The volume of requests for copies of NIA publications following the announcement of the availability of a new publication has reached 500 per day.

The NIA mailing list continues to grow and now includes over 12,000 listings, ranging from academic and research organizations to media contacts, hospitals, medical schools, and individuals.

Finally, the Information Office has maintained a clippings file of articles taken from major magazines and newspapers that directly relate to the NIA or its programs and interests. This file has had two purposes. When the Information Office conducts a publicity campaign, the clippings are a means of evaluating the success of these efforts and indicate how to modify future publicity. Furthermore, clippings are a good source of general information that IO staff can share with older people and their families when they contact the Institute for information on a specific topic.

NIA ANNUAL REPORT
OCTOBER 1, 1978 THROUGH SEPTEMBER 30, 1979
BEHAVIORAL AND SOCIAL SCIENCES PROGRAM

The Aging Research Act which provided for the establishment of the National Institute on Aging called for the support of biological, behavioral, and social research on aging. Thus, research and training in the social and behavioral sciences are an integral part of the Institute's mandate. The Program expends about 25% of the research grants budget of the Institute. While in absolute numbers or percentages, the growth of the Program since fiscal year 1975, the initial year of the NIA, may not be astronomical, there have been significant advances and trends which certainly point to considerable growth of the Program. The Institute's interest in support of behavioral research has gained greater visibility through certain activities and a broader range of scientists have been attracted to the Institute's Program. Two activities during Fiscal Year 1979 which contributed significantly to this increased visibility were the NIA-NIMH Conference on Older Women and a contract to the National Academy of Sciences, Assembly of Behavioral and Social Sciences, to organize and conduct three conferences: The Aged and Their Families, the Future Elderly, and the Biology/Behavior Interface and Aging. The Conference on Older Women concentrated on the psychological and social aspects of aging in women, focusing primarily on continuities and discontinuities in their lives. The National Academy of Sciences conferences included discussions of changes in stability of the family which are taking place and the influence of these changes for the aging; the characteristics and projected problems of the elderly of the future; and the relationship existing between biological and behavioral aspects of aging. Both these activities invited and attracted researchers not previously identified with the area of aging. Many of these investigators are expanding their research interest to include the aging processes. The staff of the Behavioral and Social Sciences program has remained extremely small throughout the existence of NIA. It has consisted of no more than three persons at any point in time, and each of these three was not devoting full time to the Program because of other duties and responsibilities within the Institute. Yet, the scope of activities of the Program is very broad, necessitating varied staff for adequate coverage. This scope extends from studies of physiological psychology through studies of factors related to service delivery to the aged. Planned staff of the Program, however, includes considerable growth. Recruitment is currently underway for an Experimental Psychologist and two additional clerical personnel to assist the one already on board. An Associate Director, Behavioral and Social Sciences, has been selected and is scheduled to come on board September 1979. While some growth of the Program can be pointed to, continued growth is dependent upon adequate staff to plan and develop various areas of research.

The following sections report on present foci of NIA support of Behavioral and social research, highlight recent findings and indicate areas where further development is required.

Social Sciences Research

Increasingly, social scientists are viewing aging in terms of a life-course perspective. Rather than considering old age as an isolated period in the lives of individuals, the life-course perspective views old age as part of a broader process of aging -- growing up, the middle years, and growing old -- which places emphasis on the influence of earlier biological, behavioral and social conditions on later ones. There is a need for research that can specify the mechanisms by which earlier life events affect later life and how developmental processes are influenced by socioenvironmental factors.

One NIA supported project is serving as the focal point in the use of the life-course perspective for interpreting the findings from existing longitudinal studies, and using the major findings of these data sets to specify the life-course perspective in more detail. The grant award has supported two topical meetings this year which brought representatives of well-known longitudinal studies together. The first meeting focused on the Michigan Panel Study of Income Dynamics and the Duke Longitudinal Study in discussions of the relationships over the life-course between people's work lives and economic well-being and their health and physical and psychological functioning. The second conference focused on interconnections over the life-course between changes in individuals' physiological/health status and their psychological/personality development, centering discussion around the Berkeley Intergenerational Study and the Baltimore Longitudinal Study. Both meetings demonstrated that focusing on specific data sets within the context of a multidisciplinary research conference is a viable means of specifying the life-course perspective in more detail and suggesting courses for future research. This project can contribute significantly to the social science literature by indicating that much can be learned about people's lives by piecing together the findings of diverse studies which lend themselves to longitudinal or cohort analyses.

A number of demographic studies relating to the older population are being supported. It is clear that the elderly population is growing. Since those who will be age 65 in the year 2020 are living now, the accuracy of projections of the numbers of future elderly is largely dependent on the accuracy of projections of mortality, particularly at the older ages. Population and mortality data from the extreme aged, especially those based on census data, have generally been considered subject to a large degree of error, particularly for nonwhites. The objective of one NIA-supported study is to measure mortality and longevity of the extreme aged (85 years or over) in the United States more accurately than through use of the official rates based on the census. Estimates of the population 85 and over in 1960 are devised through a procedure known as the "method of extinct generations" which permits the reconstruction of "extinct" population cohorts from a series of annual death statistics. Estimates of the total population by single year of age and of sex-color groups by five year age groups are then compared with the 1960 census. In general, a very close correspondence is noted among whites, but for nonwhites, the death reconstruction method yields an estimate that is 12% lower than the census tally. Other evidence such as the sample of death certificates matched with records for the same individual in the 1960 population census confirms the greater exaggeration of age in the census data.

Even though the rate of residential mobility among older people is about half the magnitude of that of the general population, approximately one-fourth of persons over 60 moved between 1965 and 1970. An NIA-supported study has identified those areas (states and counties) of the country that are gaining migrants and the areas from which these older migrants come. The study indicates that two-thirds of older movers moved within the same county of residence, one-fifth crossed county lines but remained in the same state, and one-seventh moved to another state. Florida received nearly a quarter of the nation's elderly interstate migrants. Florida's in-migration is more than twice that of California, the second largest receiver of migrants. Arizona is a distant third. New York dominates the list of states with high out-migration of persons over 60, sending nearly twice as many migrants as California and Illinois, the next largest states.

Despite the fact that interstate migrants represent a relatively small group, their patterns of migration have a potential impact on both the state of origin and the state of destination. In general, interstate migrants are not a random subsample of all older persons, but tend to be over-representative of higher SES groups. Their migration affects the area of origin by leaving a less well-off older population, and the area of destination by adding to the size of the older population.

About twenty percent of the national migrating older population in 1970 returned to their state of birth. Compared to other migrants, those who returned to their state of birth were less well educated and had lower incomes. Aged "return migrants" were more often not married than other migrants. Also, the pattern of return migration is more common among blacks than whites.

Although mortality is generally viewed as the most stable, effectively controlled, and therefore, least problematic factor in forecasting future population size, there recently has been considerable movement in age specific (and total) mortality rates. An Institute-supported study, the objective of which is to evaluate and apply multiple cause mortality statistics to the analyses of mortality differentials across selected demographic variables (race, sex, age), has looked specifically at the black-white mortality cross-over. These studies, using population data adjusted for known race misclassification and enumeration problems, found that sex specific racial mortality rate cross-overs existed for different major causes of death and that each major cause of death exhibited a different age at cross-over. Also, it was found that the trend in the cause specific mortality probabilities which led to the cross-over at advanced ages began early in life, about age 45. Furthermore, a number of epidemiological and clinical findings were found to be consistent with the observed cause specific cross-overs. These studies have been conducted both for multiple cause data for 1969 and for underlying cause data for 1962-1976.

Studies using the multiple cause mortaility data show that chronic degenerative diseases are reported more frequently as an associated (non-underlying) cause of death at advanced ages. This suggests that analysis of mortality changes using only underlying cause mortality data would be misleading and that differential biases would exist across demographic variables.

Wording of questions on public opinion surveys is known to influence responses. Also found is a trend for older people to give more favorable responses to questions about life circumstances than is warranted by objective conditions. The discrepancy between respondent's and observer's views may indicate genuine satisfaction on the part of older people with conditions those in other age groups deplore. Alternatively, this discrepancy may indicate ego defense, or attitudes developed on the part of persons in unsatisfactory circumstances they cannot change. Research is ongoing to assess the influence of question and response format upon response of a representative sample of Californians 60 years of age and over. The central interest was the effects of question orientation to factual description of reality vs. orientation to the respondent's ability to cope with that reality. The results indicate that positive response bias is related to question format, being least affected in response to objective description and most affected with respect to the respondent's ability to cope with the described aspect of reality.

There are policy implications of this finding. Insofar as studies continue to report that the elderly are satisfied with their life circumstances, it would be more difficult for society to act upon the assumption that older people are dissatisfied.

A variety of large-scale surveys have been conducted on the extent of illiteracy and functional competence in the U.S., but none have included a sizable representative sample of persons over the age of 65. Consequently we know little about the literacy or functional competence levels of the aged.

Beyond the psychological and social barriers that may exist regarding the aged applying for needed health and social services, there are severe reading difficulties that arise from the aging process itself (declining eyesight, poor attentional mechanisms, etc.), from a lack of formal education, or from the sheer complexity of the reading materials themselves. An NIA investigator has found that the elderly suffer great difficulties in completing the forms necessary to acquire needed health and social services. In many cases, they need the assistance of another individual in order to complete the forms which is not an efficient way in which to accomplish this task. As a part of this study assessing reading demands upon the aged, a comprehensive sample of application forms for health and income-related programs have been collected and examined (Social Security, Supplemental Security Income, Public Assistance, Food Stamps, Medicaid and Medicare). Documents relating to these services were chosen because it is only through these documents that aged persons can gain access to the particular service, and it is these services that are most frequently used by aged persons. It was found that there are a large number of rather difficult words contained in application forms for Federal social programs. It was also found that there is relatively little commonality among the most frequent unfamiliar words in the documents sampled. This finding is important since this rather low degree of commonality across forms produces the need for a large basic vocabularly for a person when completing the various applications. Consequently, the person would have to possess well-developed literacy skills to achieve this without any external assistance. In order to ensure the participation of all eligible members of the population regardless of level of literacy, efforts are necessary to provide instruction relevant to the development of recognition and understanding of the basic vocabularly of these application procedures and/or simplified documents should be prepared.

Another research project in the general area of reading ability seeks to determine the extent to which the design of information displays affects the performance of older persons in tests involving their visual capabilities and includes a series of studies of legibility of both printed and optically projected material. The studies examine the effect of type-style, size, and layout of printed materials on reading speed, ease of reading and interest in written materials. Higher reading speed has been found to be associated with a particular type style (usually found in school books) among the aging. An unexpected result indicates that ease of reading is not enhanced by increasing the type size. In fact, very large type face results in judgements of greater difficulty of reading than smaller type size; a 12-point type was judged easiest to read. Interest in a written passage seems to be a function of both type size and type style, as well as the content of the passage.

In order to maintain a successful and self-sufficient life-style, old people, like all members of society, are required to read and grasp the meaning of a great deal of printed and optically projected material. Some people may be judged intellectually deficient because they fail to grasp the meaning of a piece of writing or printing, when the lack of comprehension may be the result of a sensory failure rather than an intellectual failure.

Although the recent amendment to the Age Discrimination Act of 1967 accompanied by continued high inflation may curtail the trend toward higher retirement rates somewhat, the growth of the elderly population and widespread retirement will continue to affect the lives of the individuals and society as a whole. Yet, neither the factors that influence the retirement decision nor factors influencing psychological and social adjustment are well understood. Whether the economy will be able to sustain an economically dependent aged population as the post World War II baby boom cohorts enter the ranks of the elderly early in the next century, remains a controversial question. Several recently funded studies of retirement should help to fill this gap in our knowledge. These include:

A study on the retirement decision which is examining the effect of the earnings test under Social Security and the amount of potential Social Security benefits on the labor supply (labor force participation, number of hours worked per week, and number of weeks worked per year) of members of a recent cohort of older persons using data from the Social Security Administration's Longitudinal Retirement History Study.

Another study explores the decision to migrate upon retirement among a sample of midwestern pre-retirees. An ultimate aim of the study is to aid in the prediction of future migration flows of elderly persons from the Midwestern states.

A macro-sociological study of retirement is examining retirement rates in 65 countries. The investigator proposes to test the hypothesis that societal level demographic and economic factors account for differences in retirement rates.

The impact of the retirement decision on the individual, using data from the Longitudinal Retirement History Study, to examine the effect of retirement and associated life changes on individuals' health status and health services utilization. The investigator hypothesizes that the presence of social support networks will mediate any declines in health and use of health services.

Behavioral Sciences Research

Much of the research in the behavioral sciences program continues to be concerned with the identification and specification of cognitive and intellectual changes that occur with aging.

It is widely hypothesized among gerontologists that the elderly have more trouble paying attention and concentrating than do younger individuals. Yet this view has received no documentation from studies that measure brain activity directly. NIA-supported research has resulted in the development of a brain wave test of attention problems in older people that appears to assess the resiliency of their brain functions related to the switching of attention. The test procedure involved asking both young and older individuals to transfer their attention quickly back and forth from a simple to a difficult task. When required to do two things at once (difficult task) both young and older groups show decreases in the size of a brain potential known as contingent negative variation or CNV. This reduction in CNV is called a distraction effect. A most interesting thing happens when one of the two tasks is unexpectedly omitted and only one task remains to be performed (easy task): young people show a supranormal elevation in CNV size -- called the CNV rebound -- whereas older individuals do not. CNV rebound appears to represent the brain's "acknowledgement" that a difficult task (doing two things at once) has been replaced by a simpler task (doing only one thing). The fact that the elderly show such a blunted CNV rebound suggests that the aging brain "behaved" during the simpler situation as if still working on two tasks. Diminished CNV rebound in the elderly is direct evidence that the aging process involves a weakened ability to switch attention.

This research has also accrued substantial evidence that psychological disturbances in older people can be detected by the simple counting of their eye blinks. For example larger increases in eye blink frequencies were observed in the elderly when they were confronted with a difficult task, compared to younger individuals. Since blink rate is an accurate indicator of general psychological disturbance, eye blink frequency is viewed as a simple and reliable method for the assessment of distress in the elderly and a measure of potential practical importance.

In a study of the effects of hypertension on cognitive functioning among two age groups, an investigator has found that persons with hypertension performed less well than a matched group of persons without hypertension on several tests of cognitive functioning (intelligence tests and several neuropsychological tests). However, in this cross-sectional study the negative effect of hypertension on cognitive functioning was greater among the younger people than among the middle-aged. Older subjects performed less well than younger subjects for the normotensive groups but not for the hypertensive group; the younger hypertensive group performed less well. Medicated hypertensive subjects performed slightly poorer than nonmedicated hypertensive subjects but this difference was not statistically significant and disappeared altogether when subjects were equated for blood pressure values at a time when they were off all medication.

Older persons have been found repeatedly to be less effective at solving problems than younger persons. This may be due to differences in basic thought processes such as reasoning from available evidence or planning and executing strategies for gaining information. However, one investigator working in this area reports results which provide little support for the hypothesis of "regression" with age in problem-solving styles. The results deomonstrate similarity between the elderly and middle-aged in cognitive approaches to problems, provided that health status and education levels are comparable. The research is aimed at identifying patterns of concrete and formal thought usage across a series of novel and familiar tasks presented to middle-aged and older men and women. Abstract analytical problem-solving styles were most often used by middle-aged men, whereas operational and intuitive styles were most common among women. High intra-individual variability suggests that these trends reflect differing cognitive strategies or approaches rather than problem-solving competence per se. Another study compares the utilization of information about problem-solving in younger, middle-aged, and older subjects. Through the use of a problem familiar to almost everyone, making a product selection as a consumer, this research indicates that younger subjects asked for one-third as many pieces of information as the middle-aged or older subjects, though all groups worked at the same rate. The experimental design varied the complexity of the task and when the number of alternatives was increased the number of questions increased, but by less than a factor of two. The manipulations that increased complexity, however, did not interact with age. Results show no age differences in utilization of information about solutions to problems in subjects selected from a homogeneously well-educated population.

Changes in cognitive functioning are common reactions to transient stresses. This relationship with chronic stress, however, is not well established. While stress is traditionally implicated in the etiology of psychoneurosis, mild psychopathological disturbances may become second order stresses, consequently exerting greater impact on adaptation and general well being. This NIA study was undertaken to determine if the presence of such chronic stressors would be related to differences in intellectual functioning in successive life stages. On the basis of medical health records, subjects were classified as to presence or absence of psychoneurosis over a 14 year period prior to testing. Results indicate that cognitive performance could not be predicted on the basis of mild psychopathology during any life stage.

Suitable measures of stress and coping processes are badly needed to advance our knowledge of aging and adaptation. The objective of an ongoing project is to systematically describe stress, coping, and emotional patterns in the daily lives of "normal functioning" people. As a part of this research project two sub-scales have been developed: one measures problem-focused coping and the other emotion-focused coping. People vary in the pattern of coping (problem vs. emotion-focused) displayed from one stressful encounter to another. In a group of subjects it was found that work related stresses tended to elicit problem-focused coping and that health and family related stresses elicit emotion-focused coping. The appraisal of a stressful situation as one in which little or nothing can be done tends to elicit emotion-focused coping. Women were not found to differ from men in their patterns of coping, except in the use of problem-focused coping which predominates for men at work. It is hypothesized that the elderly might use more emotion-focused forms of coping because of increasing incapacity and helplessness.

A longitudinal study which began in 1968 and focused on 502 participants aged 45 to 69 at time of sample selection, has examined the way in which the subjects have adapted to stresses of life-cycle changes. In general, those with low-psychological resources, that is, low intelligence and high anxiety, were more likely to experience potentially stressful events (illness, widowhood, retirement, last child leaving home) during the study. Of the five major events requiring social and psychological adaptation, it was found that retirement had more effect than any other single event. That is, it had negative impact on a number of variables such as life satisfaction, affect balance, and feelings of usefulness. There was, however, no relationship found between retirement and decrease in health. Major medical events and widowhood were not found to be particularly stressful. The results indicate that most of the potential stressors have little effect, and that many of the occurrences people believe are particularly stressful in old age are not borne out by the study.

NIA ANNUAL REPORT

Report of Biomedical Research and Clinical Medicine Program

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The Basic Aging Program (BAP), Biomedical Research and Clinical Medicine (BRCM), is responsible for NIA extramural research support in five areas (see Figure 1). A unifying theme of BAP is the support of research in which models, cellular, organismic and simulation, are used to understand mechanisms of aging. This basic research is recognized as in support of research on experimental mammals and man. BAP has primary BRCM responsibility for cell biology, genetics, computer modeling of aging and dermatology. In cellular aging, BAP is responsible for research on cells that are genotypically and/or phenotypically distinct from the normal, counterpart cells of the parent organism. These studies emphasize cell biology, and are comparative. This concept recognizes the research opportunities offered through contrasting, and sometimes less complex, cell-growth circumstances; however, it further serves to acknowledge the limitations imposed as a consequence of genetic and/or phenotypic remoteness from normal cells in the parent organism. This remoteness, and the uniqueness of cell growth conditions, must be appreciated in experimental design, and especially, data interpretation. The cellular aging which fits within this concept is, 1) that conducted in vitro independent of the parent organism, 2) in vivo research where the cells under study are introduced into a host organism through transplantation, or chimeric manipulation, and 3) genetic mosaic research. Research in these three classes is supported through the BAP subprogram, Genetics and Cellular Aging.

In comparative aging at the organismic level, research is pursued with invertebrates, plants, and prokaryotes. The approach is holistic, often with each type of organism under study pursued with appreciation for its full biological complexity through the complete life-cycle. In most situations, these organisms are utilized for their characteristics favorable to genetics research. The animal and plant subjects of this research are the most remote, genetically, from experimental mammals and man. Because of this genetic and evolutionary distance, the research is recognized as comparative. As with the cellular aging research within BAP, extrapolation of data to interpretation of aging processes in higher animals and man must be done with caution. Advances with comparative studies are expected to provide testible hypotheses in experimental mammals. Research on these lower organisms is supported through BAP subprograms of Genetics and Comparative Aging.

The simulation of biological processes of possible significance to aging is supported within BAP. The scope of this research encompasses the entire range of organisms from the most simple through man. It may address a complete organism, or a constituent biochemical process. It covers the allied fields of population genetics and quantitative genetics. The research encouraged is both data driven, (the simulation model tied directly to experimental data) and theoretical (remote from experimental animal data). Not unlike the constraints on interpretation of the cellular and lower-organism studies described above, simulation must be appreciated as an abstraction of biological activity, and therefore is also comparative in nature.

Research on the aging of skin, from molecular and cellular studies through physiological and clinical research, is supported within BAP. The immediate focus of this research, unlike other BAP activities, is directly on human health. The objectives range from the biology of skin aging to the epidemiology, etiology, diagnoses, treatment, and prevention of skin problems of the aging and the elderly. Beyond these objectives, however, gerontological and geriatric dermatology offer unique opportunities to pursue cellular aging as a model of aging in other tissues and organs, and to test concepts of the relationship between *in vitro* and *in vivo* cell biology. In these contexts, research on skin aging supports the unifying theme of BAP: a heuristic contribution to the total NIA endeavor to improve human health. This research is supported in the BAP subprogram of Dermatology.

The above outlined subprograms of BAP carry the Program's primary *research* responsibility. In support of this research the subprogram, Genetic and Cellular Resources, serves in the supply and characterization of biological research materials. The efforts of this subprogram, such as the supply of highly-characterized cell-lines, are among the most visible of BAP programming undertakings to the NIA constituent scientific community.

In summary, BAP serves a primary function through fundamental research in the support of the other BRCM programs, both of which are oriented to gerontologic and geriatric studies primarily on mammals and man. Much of the research supported through the Molecular and Biochemical Aging Program, and the the Biophysiology and Pathobiology of Aging Program has a supportive counterpart within BAP.

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National Institute on Aging

Biomedical Research
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Clinical Medicine

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Gerontology
Research
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Basic Aging
Program

Molecular and Biochemical
Aging Program

Biophysiology and Pathobiology
Aging Program

Genetics and Cellular Aging	research on cultured and transplanted cells, chimeric and genetic mosaic animals
Genetics and Comparative Aging	research on invertebrates, plants, and prokaryote models
Theoretical Gerontology	simulation research, population and quantitative genetics
Dermatology	gerontological and geriatric dermatology
Genetic and Cellular Resources	supply and characterization of biological materials

Figure 1

GENETICS AND CELLULAR AGING, BAP Overview: The NIA support of research on the cell biology of aging is conducted in this subprogram. The support is limited to studies in which the cells are subjected to a foreign environment or one in which the cells are genetically distinguished from cells of the same type in the host or parent organism. The research addresses the cellular level. The concepts of foreign environment and genetic distinction encompass cell, tissue and organ culture, and chimeric and genetic mosaic animals. The capacity to study human cells, especially cells with differentiated characteristics, independent of the parent organism has been emerging over the past decades through the technologies of cell culture. Research on isolated cells in culture have provided remarkable access to basic knowledge of cell structure and function. Although biochemical and morphological markers serve to identify cells as differentiated, relating them to presumed counterpart cells *in vivo*, such putative relationships must be interpreted with caution. Cells in culture are usually closely identified genotypically with the organism from which they are derived. However, the circumstances of the cell culture environment inevitably cause these cells to be remote phenotypically from cells *in vivo*. With proper appreciation of the limits of cell culture systems, they do provide models for comparative studies in cellular aging. The advantages of cultured cells as models for cellular aging research include 1) access to experimentation on human cells, 2) the ready accessibility of tissue to generate cultures from surgical procedures such as circumcision, simple skin biopsies, blood samples and autopsy, 3) the ability to study cells individually or in mass quantities, 4) the relative simplicity of, and the capacity to control, the cell culture environment as compared to the *in vivo* environment, and 5) the large, highly competitive community of scientists conducting basic cell biology research on cell culture systems to understand phenomena of cell structure and function other than aging.

Through Genetics and Cellular Aging, NIA encourages descriptive studies to ascertain expressions of senescence in cultured cells, and the subsequent studies to determine the mechanisms responsible for the phenomena of senescence. Somatic cell genetics, which has contributed to knowledge of chromosomal location and function of specific human genes, is a field encouraged through this subprogram to investigate the genetic control over cellular senescence.

The transplantation of cells and tissues, including serial transplantation progressively from one animal to the next, enables observation of senescence and survival features of cells that are genetically and/or morphologically distinct from those of the host. Similarly, chimeric and genetic mosaic animals offer the means to compare, within one organism, differential expressions of cell senescence and cell survival. Chimeric animals enable observation of development and senescence of cells of different genetic heritage within the normal tissue arrangement of one animal. This is made possible through emerging technologies enabling the incorporation of genetically distinct cells into the host organism during very early embryological stages. Such chimeric animals are also obtained by combining early embryos of different genetic constitution. Genetic mosaics, on the other hand, are animals which have different phenotypic expressions of the same cell types due to alteration in genotype, or differential phenotypic expression. Research supported in Genetics and Cellular Aging ranges from a determination of control factors involved in cell proliferation, to differential expressions of cellular senescence in genetically marked cells, _in vitro_ and _in vivo_. Within Genetics and Cellular Aging there are supported 48, research grants.

Accomplishments: This BAP subprogram supports research on various aspects of cellular and molecular biology which include _in vitro_ proliferative capacity of various cell types, somatic cell genetics, cytogenetics, and DNA repair capacity of cells, structures and functions of plasma membranes and various cytoplasmic structures, and age-associated changes in the transcription and translation systems of the cell. Several discoveries in these areas with potential importance in gerontology have been made during the past year. Some of these are briefly described below.

In Vitro Life Span of Cells:

Most of the research on cellular aging uses normal fibroblast-like cells of lung and dermal origin. These cells have limited proliferative capacity in culture. The proliferative ability of these cells is inversly related to the age of the donor. The loss of _in vitro_ proliferative capacity could be an indication of cellular aging is further suggested from the observation that cells of individuals, with certain rare genetic diseases and exhibiting symptoms of accelerated aging, have extremely limited _in vitro_ life span.

Further studies on the characterization of fibroblast-like cells have been done during the past year. These include:

Clonal Analysis of Aging Human Diploid Cell Culture, (P.I.: Dr. James R. Smith; AG-0038-03).

The overall objective of this project is to evaluate critically various hypotheses of cellular aging by using human diplod fibroblast and other diploid cell culture model systems.

A human embryonic lung fibroblast-like cell line (Flow 2000) at different passage levels was cloned and subcloned and the frequency distribution of proliferation potential of cells was determined. It was observed that:

(1) cells in clones are more homogeneous in proliferative potential than cells in the mass culture.

(2) cells in clones exhibit considerable variability in their proliferative potential with time in culture. Clones from single cells, which completed 16 doublings, contained cells that underwent zero to 33 additional population doublings.

(3) the distribution of proliferative potential among the subclones isolated from single parent clones was distinctly biomodal; 10-40% of the subclones had proliferative potentials similar to the remaining in vitro life span of the parent clones.

As the parent clones approached the end of in vitro life span, the number of cells with greater doubling potential decreased. The proliferative capacity of sister cells from 337 mitotic pairs has been followed and it was observed that the two sister cells from a single mitotic event could differ by at least eight population doublings. In 40% of cases, if one sister failed to divide, the other one also did not divide. However, when one sister divided 2-7 times, the other one exhibited variable doublings, especially when cells were near the end of their in vitro life span.

The high degree of variability in division potential of cells within a single clone and between sister cells is compatible with the following hypotheses: 1) fixed, finite number of divisions per cell, 2) in vitro cell differentiation and 3) direct changes in the nuclear genome. These data are qualitatively compatible with the hypothesis that suggests a mechanism involving unequal inheritance of nuclear or cytoplasmic genetic or epigenetic elements between daughter cells.

Publications:

Smith, J.R. and K.I. Braunschewiger. 1979. Growth of human embryonic fibroblasts at clonal desity: concordance with results from mass cultures. J. Cellular Physiol. 98: 597-601.

In Press (Submitted)

Smith, J.R., Pereira-Smith, O.M. Braunschewiger, K.I. and R Whitney. 1979. Colony size distribution: a quantitative tool for the study of in vitro proliferation potential. II. Correlation with in vitro proliferation potential of human diploid fibroblasts. Exp. Cell Res. (submitted).

Smith, J.R. and R.G. Whitney. 1979. Intraclonal variation in proliferation potential of human diploid fibroblast: evidence for a stochastic mechanism for cellular aging. Science (submitted).

Clonal Analysis of Aging Human Diploid Cell Cultures. (P.I.: Dr. James R. Smith; R01-AG-00338-02)

The colony size distribution technique has been used in recent years to determine *in vitro* proliferative capacity of human fibroblast-like cells (Smith et al. 1978). However, little is known whether the growth potential of cells at clonal densities is similar to that of mass cultures. This question has been dealt with in a recent study by Smith and Braunschweiger with WI-38 and IMR-90 fibroblast-like cells. Their results show that clonal and mass cultures are similar with respect to their attachment to the culture plate, proliferative capacity and population doubling time.

Publications:

Smith, J.R. and K.I. Braunschweiger. 1979. Growth of human embryonic fibroblast at clonal density: Concordence with results from mass cultures. J. Cell Physiol. 98(3): 597-602.

Smith, J.R., O. Pereira-Smith and E.L. Schneider. 1978. Colony size. distributions as a measure of *in vivo* and *in vitro* aging. Proc. Natl. Acad. Sci. (U.S.A.) 75: 1353-1356.

Clonal Senescence of Somatic Cells. (P.I. Dr. Thomas Norwood; AG-00257).

Recently Drs. Martin, Norwood and their associates compared the growth patterns of primary clones and serial subclones of HeLa cells and of diploid human firboblast-like cells. They observed that clones and subclones of HeLa cells, similar to fibroblast-like cells, exhibit great heterogeneity in growth patterns and bimodality of growth distribution. Serial passaging of clones of HeLa cells of high proliferative capacity led to attenuation or gradual decrease of growth potential.

However, the extent of such attenuation was much less in the case of Hela cells, suggesting at least one possible basis for the differences in long-term growth potential between these two classes of cell lines. The loss of growth potential of some cells of the neoplastic HeLa cell lines could be due to the following reasons: 1) segregation of chromosomally aberrant types of cells that cannot divide or have limited division capacity; 2) the reversion of the neoplastic phenonmenon by clonal senescence; and 3) unbalanced growth associated with unequal distribution, loss or impairment of vital cell organelles.

Publication:

Martinez, A.O., T.H. Norwood, J.W. Prothero, and G.M. Martin. 1978. Evidence for clonal attenuation of growth potential in HeLa cells. In Vitro 14: 996-1002.

Clonal Senescence of Somatic Cells. ('P.I.: Dr. Thomas H. Norwood; AG-00257).

The purpose of this project is to determine the mechanisms whereby cultures of normal human and other mammalian cells taken from different tissues of the body, regularly appear to have limited life span, whereas cultures of cells having certain properties of cancer cells, seem to have indefinite life spans. The problem is of significance in that the in vitro behavior of the cells may serve as models for the aspects of aging which relate to the ability of cells to continue to regenerate and to closely regulate each other's reproduction.

Newly developed methods for fusing cells in culture have been used for analyzing the phenomenon of "in vitro aging." For example, test crosses have been made between cells from cultures having comparatively long life spans with cells from cultures showing comparatively short life spans; these result in hybrids giving intermediate life spans. Such results are interpreted as evidence against one variety of mutation theory for the mechanism of in vitro aging. A variety of evidence suggests that most mammalian cells stop dividing in vitro; they undergo "terminal differentiation" towards specialized cell types which no longer divide. The rates at which such processes occur could have relevance to those aspects of aging which relate to insufficient or excessive regenerations of cells or tissues.

Publications:

Martin, G.M. 1979. Proliferative homeostasis and its age-related aberrants Mechanisms of Aging and Development. 9: 385-391.

Hoehn, H. Bryant, E.M., and Martin, G.M. 1978. The replicative lifespans of euploid hybrids derived from short-lived and long-lived human skins fibroblast cultures. Cytogenet. & Cell Genet. 21: 282-295.

In recent years, attempts have been made to culture and study the lifespan of various tissue and organ specific cells. The use of such differentiated cell cultures provides and excellent opportunity to investigate mechanisms of age-associated alterations in differentiated functions expressed by cells in vitro. Recent studies by NIA grantees on differentiated cell culture include:

Program Project in Cell Biology of Aging: (P.I. Dr. Richard Ham, P01-AG-00374-05).

Drs. Peehl and Ham have recently succeeded in culturing keratinocytes of foreskin without the fibroblast feeder layer. Keratinocytes can be grown in the medium, conditioned with irradiated 3T3 cells. In this conditioned medium keratinocytes underwent 30 population doublings and synthesized keratin. They have also been succeeded in culturing keratinocytes in unconditioned medium supplemented with hydrocortisone and bovine pituitary extract. In another study Drs. McKeehan and Ham observed that some phospholipids which remain associated with fetal bovine serum proteins, promote human fibroblast-like cell proliferation. The ability to maintain keratinocytes for an extended period of time in culture would allow use of these cells in studies on age-associated loss in differentiated functions.

Effect of Environment on the Aging Process (P.I. Dr. Barbara Gilchrest: Dr. Gilchrest is a co-investigator of the Program Project No. P01-AG-00599-03; P.I. Dr. John W. Rowe).

The objective of this project is to determine whether chronic sun-exposure accelerates skin aging, as judged by morphologic, behavioral, and biochemical criteria for cells in tissue culture. Attempts will also be made to see if cultured keratinocytes would be good materials for cellular aging research. This project is the only cell-culture study in this Program Project (primary assignment to MBAP). This study, in which cell culture provides a model for comparative studies in skin aging, is supportive of the BAP Genetics and Cellar Aging and Dermatology subprograms.

Keratinocytes from sun-exposed skins from adult males of 20-80 years were cultured. In vitro population doubling levels (PDL) were found to be lower for keratinocytes from exposed than from unexposed skins. Further decrease in PDL was noted with increasing donor age and severity of clinical aging changes in the skin. Skin biopsies were used to determine melanocytes (dopa positive) density. The density of melanocytes in the sun-exposed skin was twice as great as in the non-exposed skin. In addition, a striking decline in melanocyte density was found to occur with age; this observation is similar to those in which a decrease is seen in the density of dopa positive melanocytes in human skin throughout the adulthood. Therefore, an increase in melanocytes density in the sun-exposed skin is probably not an indication of sun induced acceleration of the aging process. The study proposed by Dr. Gilchrest attempts to identify and quantitate environmental factors which accelerate many aspects of skin aging. Age-associated changes are most conspicuous in the skin and are probably most preventable as well.

Publications:

Gilchrest, B.A. The relationship between actinic damage and chronological aging in keratinocytes cultures of the skin. J. Invest. Dermatol. (In press).

Gilchrest, B.A., F.B. Blog, and G. Szalo. Effects of aging and chronic sun exposure on melanocytes of the skin. J. Invest. Dermatol. (In press).

Cellular Growth and Senescence in Endothelial Cells. (P.I. Dr. Elliot Levine; R01-AG-00839-02).

The study on endothelial cells is important for understanding of age-related diseases, such as arteriosclerosis and cancer. A cloned strain of bovine aortic endothelial cells from a male fetus have been established. These cells undergo 80 population doublings. With increasing population doubling level, increased polyploidy and chromosomal aberrations occur. In addition to bovine endothelial cells, endothelial cell cultures of equine and pigeons have also been established. The identity of all these endothelial cells was based on their reactivity to bovine Factor VIII antibody. These cell cultures are now being used to study specialized functions as a function of donor age. These functions include the production of Factor VIII antigen and levels of angotensin converting enzyme, histidine decarboxylase.

Other differentiated cells are also being used, to a limited extent, in cellular aging research. These are canine kidney epithelial cells, liver cells, mammary cells, adrenocorticol cells, salivary gland secretory cells, lymphocytes and neuroglial cells.

Somatic cell hybridization:

The use of somatic cell hybridization techniques in cellular aging research has provided the following information during FY 79:

Growth Regulations: Senescent vs. Non-senescent Cells. (P.I.: Dr. Gretchen Stein; AG-00947-01).

This project deals with the mechanisms of cellular aging, and regulation of DNA synthesis in particular, in human diploid cells (HDC) in culture. Previous studies in other laboratories have shown that in somatic cell hybrids, between young and senescent HDC, the senescent HDC inhibits DNA synthesis in the young HDC nuclei (Norwood et al. 1974). DNA synthesis is induced in the senescent HDC cell nuclei when they are fused with HeLa and SV-40 transformed cells (Norwood et al. 1975). However, Stein and her colleagues observed that neoplasticity per se does not account for the induction of DNA synthesis in the fused senescent HDC. The fusion of the senescent HDC with transformed T98C human glioblastoma cells or RK13 rabbit kidney cells does not induce DNA synthesis in the senescent cell nuclei. Unlike HeLa and SV-40 transformed cells, T98C, RK13, and young HDC remain at the G1 stage during the stationary phase. However, the absence or presence of the normal stationary G1 phase does not seem to account for the difference in the ability of transformed cells to induce DNA synthesis in the senescent cell nuclei. Fusion of senescent HDC with SUSM-1 cells (chemically transformed human cells) or F98 glioblastoma cells, which lack normal stationary G1 phase, does not lead to the induction of DNA synthesis in the senescent cell nuclei. Dr. Stein and her associates speculate that the mode of transformation (i.e., virus vs. chemicals) is an important factor for the induction of DNA synthesis in senescent nuclei, present in heterokaryons.

The cell fusion studies of Stein and her associates further show that in the T98G and senescent HDC heterodikaryons, the entry of the T98G nucleus into the S-phase is inhibited. However, if the T98G cells are at the S-phase at the time of fusion, DNA synthesis continues in these nuclei in heterodikaryons. Similar results were obtained from young HDC and senescent HDC heterodikaryons. Thus, it appears that the senescent HDC which are primarily at the G1-stage, contain a factor(s) which inhibits the G1 cells from entering into the S-phase or the DNA synthetic phase.

Selected Publications:

Yanishevsky, R.M. and G.H. Stein, "Entry into S-phase is inhibited, but ongoing DNA synthesis continues in young human diploid cells (HDC) fused with senescent HDC," J. Cell Biol. 79:398b (1978).

Stein, G.H. and R.M. Yanishevsky, "Entry into S Phase is inhibited, in two immortal cell lines fused to senescent human diploid cells. Exper. Cell Res., in press.

Nucleo-Cytoplasmic Control of Aging and Malignancy. (P.I.: Dr. Audrey Muggleton-Harris; R01-AG-01212-01).

Dr. Muggleton-Harris has developed techniques for nuclear transfer and fusion of individual viable nuclei into cells. She has obtained experimental binucleates by fusing nuclei from normal fibroblast, SV40 virus-transformed fibroblast and HeLa carcinoma cells with individual diploid fibroblasts which have undergone their total life span or population doublings. Binucleates thus formed, replicate and form clones of cells with limited life span. These results suggest that the senescent Phase III fibroblast-like cells retain their ability to divide under proper conditions. However, little is known at present about the factors that control the division of these cells.

Publications:

Muggleton-Harris, A.L. and M. Palumbo. 1979. Nucleocytoplasmic interaction in experimental bionucleates formed from normal and transformed cell components. Somatic Cell Genetics 5: 397-407.

Gene Expression in Somatic Cells in the Aging Process, (P.I.: Dr. David Patterson; R01-AG-00029).

The long term objective of this research is to understand the genetic and biochemical regulation of purine and pyrimidine metobolism in mammalian cells including Chinese hamster cells, normal human cells and cells from patients with selected age-related diseases and growth disorders.

Abnormalities in purine and pyrimidine metabolism occur with malignancy, often considered age-related disease. Disorders of purine metabolism are specifically associated with certain types of immunologic disfunction. A progressive decrease in effectiveness of the immune system may be one of the central events associated with aging. Another key element in the aging process may be the ability of cells to repair damage to their genetic material. It is reasonable to expect that alterations in purine or pyrimidine nucleotide metabolism would lead to aberrations in DNA replication and/or repair since these molecules are required for these actions. In addition, certain purines, such as caffeine, have been shown to inhibit repair of radiation-induced damage in DNA of mammalian cells. These findings strongly suggest that any complete understanding of DNA damage repair in mammalian cells will require an understanding of the regulation of purine and pyrimidine metabolism. Further knowledge of the regulation of these pathways may be necessary to better understand immune system function, malignancy, and the mechanisms of DNA damage repair. It is necessary to understand each of these processes to evaluate properly the role each might play in the aging process.

Thus, a study of purine and pyrimidine metabolism regulation, which appears to be of great importance in these systems, offers a rational and unifying approach to the study of many potential components of aging. If the genetic-biochemical approaches used here are successful, they may be employed to understand biochemical and genetic regulation of other metabolic and developmental processes important in aging.

The following mutants of Chinese hamster cells, defective in purine and/or pyrimidine metabolism, were isolated:

1. Mutants defective in pyrimidine biosynthesis

2. A mutant showing a specific requirement for one purine, (adenine), only in the presence of non-toxic amounts of another purine, (guanine)

3. Several mutants which will not grow at normal temperatures (37°C) but can grow at lower temperatures (34°C)

4. Mutants lacking enzymes allowing utilization of purines added to the cell growth medium. Investigators devised a new procedure for selection of hybrid cells, and were able to achieve direct confirmation of the biochemical defects in several purine-requiring mutants.

Publications:

Tu, A.S., and Patterson, D. Characterization of a guanine-sensitive mutant defective in adenylosuccinate synthetase activity. Journal of Cellular Physiology (in press).

Liskay, R.M., and Patterson, D. A selective medium (GAMA) for the isolation of somatic cell hybrids from HGPRT and APRT mutant cells. Cell Genetics and Cytogenetics (in press).

Liskay, R.M. and Patterson, D. Selection of somatic cell hybrids between HGPRT and APRT cells. Methods in Cell Biology (in press).

Cytoplasmic Structures and their Functions in Cultured Cells:

Normal cell function involves co-ordinated activities of the cytoplasm and nucleus. Attempts are being made to find out to what extent such a co-ordination is affected during cellular aging. During the past year NIA grantees have made two fundamental discoveries relating to the structure and function of the cell cytoplasm. These are briefly described below.

Program Project in Cell Biology of Aging. (Co-Investigator: Dr. Keith Porter; P01-AG-00310-05. P.I. of this Program Project is Dr. Richard Ham).

The microtrabecular lattice structure of the ground substance of the cell is one of the most recent significant discoveries in cell biology made by Dr. Keith Porter and his colleagues at the University of Colorado, Boulder The use of high voltage electron microscopy of whole WI-38 cells led to the discovery of this structure. The sterio-images of cells revealed close association of this structure (3-6nm. strand) of the ground substance with microtubules, microfilaments, endoplasmic reticulum, and polysomes. This structure of the ground substance of the cell is called microtrabecular lattice or mesh. The microtrabecular lattice is now seen in other cells, including the protozoan, Paramecium. This protein-rich structure seems to be involved in the maintenance of cell shape and movement of various cytoplasmic structures. A comparison of cytoplasmic organelles is now being made in early and late passage WI-38 cells. Dr. Porter's group has also compared the morphology of intermediate and late passages WI-38 cell cultures, using scanning, conventional transmission and high voltage electron microscopy. Their results reveal a considerable intraculture morphological heterogeneity of cells.

Dr. Porter is a member of the National Academy of Sciences, and he is world renown for his work on the membranes and cytoskelton systems of the cell.

Publications:

Wolosewick, J.J. and K.R. Porter. 1977. Observations on the morphological heterogeneity of WI-38 cells. Amer. J. Anat. 149: 197-225.

Wolsewick, J.J. and K.R. Porter. 1979. Microtrabecular lattice of the cytoplasmic ground substance. Artifact or reality. J. Cell Biol. 82(1): 114-139.

Subcellular Structures and Synthesis and Intracellular Transport of Proteins. (Co-Investigator: Dr. David Sabatini; P01-AG-00378. The P.I. of this Program Project is Dr. Vincent Cristofalo).

Dr. Sabatini and his associates at the New York University Medical Center observed that different types of enveloped-viruses bud asymmetrically from either the apical (influenza virus WSN strain, SV5 and Sendai) or basolateral (VSV) regions of the plasma membrane of cultured canine and bovine epithelial cells. These virus-infected cells provide an opportunity to study mechanisms by which different proteins are directed to specific regions of the plasma membranes. Rodriguez Boulan and Sabatini suggested that the membrane polarity is established before budding of the virus particles.

Dr. Sabatini has made significant contributions to the function of endoplasmic reticulum in synthesis and transport of secretory proteins. His present studies on the structure, function, and biogenesis of cellular membranes are of considerable importance in our understanding of age-associated changes in membrane function.

Publications:

Rodriguez, Boulan E., and D.D. Sabatini. 1978a Asymmetric budding of viruses in epithelial monolayers: a model system for the study of epithelial polarity. Proc. Natl. Acad. Sci. 756: 5071-5075.

Rodriguez, Boulan E. and D.D. Sabatini. 1978b. Asymmetric production of viruses by epithelia. Fed. Proc. 37: 1172.

Functional Activities of Cultured Cells:

Changes in various biochemical and physiological parameters during cellular aging are being studied by several NIA grantees. Results of some of these studies are summarized below.

Fibroblasts and Connective Tissues in Development. (P.I.: Dr. Paul M. Gallop; P01-AG-00376-05).

Considerable progress has been made in this Program Project during the last few years. Some of the findings include:

a. A decrease in the activity of the X-linked enzyme HPRT, as compared to that of the autosomal APRT, with an increase in the age of IMR-90 cells in culture. Thus, it might be possible to estimate the age of cell cultures on the basis of HPRT/APRT activities.

b. A correlation seems to exist between the decrease in the X-linked processes and certain age-associated diseases, such as gout and the loss of receptors to tetesterones (which caused feminization in some aging males).

c. High levels of cholesterol are detected in cells aging *in vitro*. A similar situation may exist *in vivo*. This could lead to an increase in the serum cholesterol level of older people.

d. Vitamin K seems to play an important role in the synthesis of gama-carboxygluatimic acid containing proteins, such as osteocalcin. A rapid assay system has been developed for gama-carboxyglutamic acid in urine. Preliminary analysis shows high levels of gama-carboxyglutamic acid in the urine of senile amd post-menopausal osteoporosis. It is possible that the defect in vitamin K metabolism might be involved in some types of osteoporosis.

Publications:

Lian, J.B., P.V. Hauschka, and P.M. Gallop. 1978. Properties and biosynthesis of a vitamin K-dependent calcium binding protein in bone. *Fed. Proc. 37*:2615.

Lian, J.B. and P.A. Friedman. 1978. Formation of gama-carboxyglutamic acid in bound microsomes. *J. Biol. Chem. Communications*, 253:6623-6626.

Hauschka, P.V., and M.L. Reid. 1978. Timed appearance of a calcium-binding protein containing gama-carboxyglutamic acid in developing chick bone. *Devel. Biol. 65*:426-434.

Hauschka, P.V. and M.L. Reid. 1978. Vitamin K-dependence of a calcium-binding protein containing gama-carboxyglutamic acid in chicken bone. J. *Biol. Chem.* 253:9063-9068.

Torrelio, B.M. and M.A. Paz. 1978. Increased phosphoribosyl pyrophosphate synthetase activity in fibroblasts of hypoxanthine-guanine phosphoribosyl transferase deficient patients, *Biochem. Biophys. Res. Commun.* (In press).

Cell Surface Function and Cellular Aging. (P.I.: Dr. Gib DeBusk; R01-AG-00194-04).

Dr. DeBusk and his associates observed an age-associated alteration in amino acid transport across the plasma membrane of WI-38 cells. They reported a decrease of about 50% in transport via the L-system (transport of amino acids with aromatic and with sizeable aliphatic side chain) between the 17th and 34th passages. In contrast, a slight increase in the A-system (transport of amino acids with short and/or polar side chains) was noted with increased culture passage level. Only the amino acid transport systems that are independent of Na+ seem to decline with age. Studies of Dr. DeBusk's group further provided evidence for the existence of at least four additional amino acid transport systems in the WI-38 cells. Studies are in progress to determine if such systems are affected with culture age.

References:

Sullivan, Jerome L. and A. Gib DeBusk. Transport of L-Methionine in Human Diploid Fibroblasts and their variation with *in vitro* age (in preparation).

Biochemical Aspects of Aging *in vitro* and *in vivo*. (P.I.: Dr. Samuel Seifter; P01-AG-00374-06).

In one of the component projects of this Program Project, Dr. Seifter and his associates have been studying the proliferative ability and differentiated functions of human uterine smooth muscle cells in culture.

The human uterine smooth muscle cell is thought to be under exquisite endocrine control because of the cyclic changes that occur in the uterus and the changes that occur with almost predetermined certainty in the pregnant uterus. Therefore, efforts to establish several strains of human uterine smooth muscle cells have been taken with the following objectives: 1) to study how they would "age" in successive population doublings until the culture dies and 2) to determine how the differentiated expressions of that cell change with age and 3) how these changes are influenced by various pertinent hormones.

From uterine scrapings, obtained from two young women, two strains of cells (identified by various criteria as smooth muscle cells) have been prepared. These cells are diploid and have a finite proliferative capacity in culture, showing "senescence" after about 40 population doublings. The differentiated end-products of these cells were studied with special attention to the nature of the collagen produced. About 64% of the collagen secreted into the medium is Type I and the remainder is primarily Type III. In the cell layer, 74% is Type I and the remainder primarily Type III collagen. Some of the collagen is present in the procollagen (precursor) form. Uptake of creatine by the cells from the medium has been studied, and it appears that they have a concentrating mechanism for creatine.

References

Rifas, L., Fant, J., Makman, M.H. and Seifter, S. Human uterine smooth muscle cells in culture: Growth and ultrastructure. Experimental Cell Research (in press).

Wu, C.H. Rojkind, M. Rifas, L. and Seifter, S. Biosynthesis of Type I and Type III collagens by culture uterine smooth muscle cells. Archive of Biochemistry and Biophysics (in press).

Alterations in Chromatin and Nucleolar Functions during Cellular Aging. (Co-Principal Investigator: Dr. Renato Baserga; P01-AG00378. The P.I. of this Program Project is Dr. Vincent Cristofalo).

Recent work of Dr. Baserga emphasizes four approaches to the study of cellular senscence *in vitro*. In the first approach, alterations in the chromatin of aging WI-38 cells were measured by circular dichroism and by the binding of the dye, ethidium bromide. With both techniques, it was demonstrated that chromatin of mid PDL has a higher template activity than chromatin of late PDL cells. Differences in DNA binding proteins from young and old cells were also found.

A second approach utilized cell populations maintained in quiescence for prolonged periods of time. These populations showed a delayed response to serum stimulation, a decrease in the fraction of cells entering DNA synthesis, and a decrease in chromatin template activity.

The third approach, the study of isolated nucleoli, was based on the knowledge that when resting cells are stimulated to proliferate, one of the earliest responses is an increase in RNA synthesis. Most of this increase can be attributed to nucleolar RNA synthesis. A transcriptionally active fragment of chromatin which codes mainly for ribosomal RNA was isolated from HeLa cell nucleoli. This isolation procedure offers several advantages for the future study of chromatin structure and function in aging cell populations.

The fourth and last approach has utilized cells transformed by a temperature sensitive mutant of the SV40 virus. Such cells, when preincubated at a nonpermissive temperature, produce T-antigen which is rapidly inactivated. Under the same conditions of preincubation, T-antigen from cells transformed by the wild type SV40 virus was shown to stimulate synthesis in isolated nuclei. These results suggest that the T-antigen may function at the level of transcription and represent another tool with which to study alterations in proliferative capacity in aging cell populations *in vitro*.

References

Ide, T. Whelly, S. and Baserga, R. 1977. Stimulation of RNA synthesis in isolated nuclei by partially purified preparations of simian virus 40 T-antigen. Proc. Nat. Acad. Sci. 74: 3189-3192.

Floros, J. H. Chang and R. Baserga. 1978. Stimulation of DNA synthesis in frog nuclei by cytoplasmic extracts of temperature sensitive mammalian cells. Science 201: 651-652.

Surface Proteins and Cellular Senescence. (P.I.: Dr. Albert Millis; R01-AG-00697-03)

The purpose of this study is to identify and characterize cell surface components that can be used to distinguish young and old cells and to determine if cellular aging is correlated with alterations of cell surface and conditioned medium components.

The growth of normal human fibroblast-like cells in culture is dependent on their ability to attach to the substrate or the culture plate. Dr. Millis observed that old cells show decreased attachment to a substrate in comparison with young cells. More old cells attach to the substrate when a fraction containing fibronectin, procollagens and a 100,000 molecular weight protein, is included in the culture medium. Additional results of Dr. Millis suggest that the cell attachment is mediated by fibronectin and that old cells produce insufficient amount of this substance. It was further observed that some proteins released by old cells in culture have modified oligosaccharides on cell surface proteins. Human aging apparently involves altered cell to cell communication. This study examines aspects of that communication by comparison of cell surface properties.

Publications:

McCormick, Millis (1978) "The effects of proteins released by cultured fibroblasts on density dependent agglutination". Exp. Cell Res. 113, 211.

Millis et al. (1978) "Fibroblasts release surface proteins that function as Concanavalin A receptors". N.Y. Acad. Sci. 312, 439.

Kapp et al. (1979) "Variation in S phase of synchronous human cell lines". In Vitro - in press.

McCormick et al. (1979) "Direct visualization of collagen and procollagens in polyacrylamide gels", Analytical Biochem - in press.

Biology of the Aging Cell Surface. (P.I.: Dr. Robert Kelley; R01-AG-00191-05)

The purpose of this project is to contribute to an understanding of the integrated processes by which the plasma membrane and the associated external cell surface control and regulate the behavior of cells during development and aging.

Dr. Kelley observed that as human diploid fibroblast-like cells "age" in culture, they produce fewer and aberrant types of gap junctions, with neighboring cells. These structures have been implicated as low resistance pathways for cell-to-cell electrical coupling and passageways for direct intercellular exchange of low molecular weight metabilites. The alterations in membrane specializaton accompany other age-related changes in surface ultrastructure, topography, antigenicity and internal organization of the cell membrane. The decrease in the number of gap junction formation during aging leads to a reduction of metabolic communication between cells. It is likely that maintenance of normal communication between cells in necessary for normal growth and differentiation of multicellular organisms.

Publications:

Kelley, R.O., R. Azad and K.G. Vogel 1978. Development of the aging cell surface: Variation in concanavalin A-mediated intercellular binding and distribution of binding sites with progressive subcultivation of human embryo fibroblasts. Mech. Aging Develop. 8: 203-217.

Kelley, R.O., K.G. Vogel, H.A. Crissman, C.J. Lujan and B.E. Skipper 1979. Development of the aging cell surface: Reduction of gap junction-mediated metabolic cooperation with progressive subcultivation of human embryo fibroblasts. Exp. Cell Res. 119: 127-143.

Kelley, R.O. and J.F. Fallon 1978. Identification and distribution of gap junctions in the mesoderm of the developing chick limb bud: A freeze-fracture analysis. J. Embryol. Exp. Morph. 46: 99-110.

Kelley, R.O., J.A. Trotter, B.D. Perdue and C.B. Taylor 1979. Replica imaging of the cytoskeleton in progressively subcultivated human embryo fibroblasts. (submitted for publication).

Kelley, R.O and B.D. Perdu 1979. Development of the aging cell surface: Absence of gap junction-mediated nucleotide transfer into HGPRT mutants during progressive subcultivation of human embryo fibroblasts. (Submitted for publication).

Prostaglandins, Cyclic Nucleotides and Cellular Aging. (P.I.: Dr. Peter Polgar; R01-AG-00455-04)

Prostanglandins are products of certain essential fatty acids which are sequestered in the membrane system of the cell in the form of phospholipids. Responding to extracellular messages, fatty acids are released, prostanglandins are synthesized and expelled from the cell. The released prostaglandins affect the growth and function of other cells in the vicinity by influencing metabolic events within the cell membrane. Dr. Polgar has observed definite changes in prostaglandin production during *in vitro* "aging" of human diploid fibroblast-like cells (IMR-90) Senescing cells alter production of prostaglandin (PG) from PGE2 to PGF2 These cells also become increasingly sensitive to PGE2 in terms of growth. The presence of PGE2 in culture of these cells shortens their life span. Since PGF2 promotes cell growth, the shift of "aged" fibroblasts to production of PGF2 may be an attempt by the culture to promote its growth.

Publications:

Taylor, L., P. Polgar, J.A. McAteet and W.H.J. Douglas. 1979. Production by type II alveolor epithelial cells. *Biochim. Biophys. Acta*, *572*, 502.

DuComb, M., P. Polgar, and C. Franzblau. 1978. The effect of prostaglandins on the production of collagen in human embryo lung fibroblasts. *Prostaglandins, 15*, 699.

Houghton, B. and P. Polgar. 1979. The effect of hydrocortisone and prostaglandin E 2 on the final cell density achieved by human diploid fibroblasts. XIth International Congress of Biochem.

Polgar, P., W.H.J. Douglas, L. Torrecio and L. Taylor. 1979. The release of arachidonic acid and its conversion ot prostaglandins in various diploid cell types in culture. International Conference on Prostaglandins.

Polgar, P., L. Taylor, and D. Downing. 1979. Unsaturated fatty acid effect on cyclic AMP levels in human embryo lung fibrobasts, Prostglandins.

"Control of Histone mRNA Synthesis: an Approach to Aging" (P.I.: Dr. William Marzluff, Jr.; R01-AG-00413-05).

The purpose of this study is to elaborate regulatory mechanisms of histone mRNA synthesis in normal, aging and malignant cells.

Histone mRNA species from synchronized mouse myeloma cells have been isolated using the sea urchin histone plasmids covalently coupled to phosphocellulose. These RNA's are synthesized during the S-phase. A large species of RNA, obtained from isolated nuclei, hybridized with the plasmid histone H3 and H4 genes. This RNA, therefore, seems to be polycistronic. The S-phase nuclei are able to synthesize this RNA species in an *in vitro* system. Dr. Marzluff's group has observed further that the synthesis of histone H1 can occur non-coordinately from other histones. Some progress has also been made in studies on *in vitro* RNA processing, especially in RNA methylation, turnover and transport of RNA synthesized in nuclei *in vitro*. It was observed that a soluble protein extract leads to conversion of the large histone (H3 and H4) mRNA to a 9S molecule, which is then released from the nucleus.

The present study on histone mRNA biosynthesis is important in our understanding of the regulatory mechanisms of cell growth. The regulation of histone synthesis during the cell cycle may be important in the control of DNA replication.

In addition to the use of mouse myeloma cells, Dr. Marzluff proposed to study patterns of histone mRNA synthesis and processing in normal human diploid cells "aging" *in vitro*.

Publications:

Cooper, D.L. and Marzluff, W.F. 1978. Polyadenylation of RNA in a cell-free system from mouse myeloma cells, *J. Biol. Chem. 253*, 8375-80.

Brown, A. and Marzluff, W.F. 1978. Synthesis of low molecular weight RNA in mouse myeloma cells, *B.B.Acta*, 521, 662-76.

Marzluff, W.F., Pan, C.J. and Cooper, D.L. 1978. RNA synthesis in myeloma cells synchronized by isoleucine starvation, *Nucleic Acid Res. 11*, 4177-91.

GENETICS AND COMPARATIVE AGING, BAP

Overview: NIA Extramural Research involving invertebrates, plants, and prokaryote model organisms are supported in this BAP subprogram of Genetics and Comparative Aging. The basic mechanisms responsible for longevity and senescence remain unknown at all levels of life. Neither the evolution of these aging phenomena, nor the relative contributions to them of either innate determinants nor the external influences of the environment are known. In contrast, the strategies of biological structure and function in development and homeostasis, at the cellular and molecular levels, are well known. Cellular biochemistry and morphology are recognized as remarkably similar in all forms of life. This observation suggests that the processes responsible for senescence at the cellular level in any group of organisms may have important implications for all organisms. The great diversity of organisms, and extreme range of conditions under which populations of animals and plants have evolved and maintained themselves, offers the opportunity for differing bases for expressions of longevity and senescence at the organismic level. However, multicellular animals appear to have evolved from a common ancestral, single-celled organisms. This common origin offers an explanation of the remarkable similarity in structure and function of all animals at the cellular level. The extant multi-cellular descendents of a primitive cellular organism, for the most part, exhibit species specific limits to longevity, and express senescence phenomena. The evolutionary constraints which mold life span characteristics are likely, in a generalized way to be common to all animals. The cellular and molecular apparatus of all animals have very much in common. Thus, the molecular and cellular means to adapt to evolutionary pressures is relatively uniform throughout metazoan life. Mammals have a high probability of cellular mechanisms responsible for longevity and senescence. It is quite possible that such mechanisms active in mammals had their origins in organisms ancestral to mammals. In this evolutionary concept one traces mammals from a simple invertebrate origin through a continuum of increasing complexity. Fundamental knowledge of the events leading to senescence in simple organisms will provide information to guide future theory and experiments to increase an understanding of human aging. For this reason, comparative aging studies are encouraged through this subprogram. This research is undertaken with the appreciation that the invertebrates, plants, and prokaryotes that are the subject of comparative research are genetically and evolutionarily remote from man and other mammals.

The laboratory organisms used for research in Genetics and Comparative Aging often have in common several features desirable to the experimentalist. Paramount, usually, are favorable characteristics for genetic studies. These organisms, in addition to genetics value, are relatively simple, easily cultivated under well-controlled conditions, and have short life spans. The relationships between the genome and cytoplasm and the interactions between cells, tissues, and organs are particularly available to molecular dissection in these simple organisms. Populations and/or mutant lines of experimental organisms which express differential senescence and longevity, including sexual differences in longevity, could enable elaboration of the fundamental mechanisms responsible. Genetic dissection of organisms to explain senescence is difficult to achieve as senescence probably involves interactions of multiple genes. It remains, nonetheless, one of the most promising avenues of gerontology. Research through Genetics and Comparative Aging has been especially encouraged utilizing the fruitfly _Drosophila_, the laboratory nematode _Caenorhabditis elegans_, the slime mold, _Dictyostelium_, the colonial flagelate _Volvox_, and Protozoa. The research encompasses molecular genetics through the general biology of aging at the cellular, organismic and population levels. Commonly, these organisms are the subject of a broad range of highly competitive research, of which gerontology is but a small part. As most such research is on developmental biology, these fields focusing on specific laboratory organisms are excellent for expansion to encompass the interests of the NIA. Knowledge of genetics control over maintenance of fitness and longevity is of major importance to the NIA mission. The data necessary to this end appear most readily accessible through these invertebrate models. Within Genetics and Comparative Aging there are 36 research grants.

Accomplishments: Much of the research in the subprogram on Genetics and Comparative Aging utilizes the laboratory nematode _Caenorhabditis elegans_. These nematodes are hermaphrodite and they have a short life cycle: about 2 days from fertilization to adulthood. They live for about 9-10 days. These organisms are easy to culture in the laboratory. Their anatomy in simple and cell lineages from fertilization to 200 cell stages have been followed. The entire neuroanatomy of the organism is known. Many mutants have been obtained and genes are mapped. About 80,000 genes equivalent of DNA and only about 2000 essential genes per genomes are reported. Some of the studies of NIA grantees utilizing nematodes include:

A Genetic Analysis of Aging (P.I.: David H. Mitchell; R01-AG-00952-01).

The purpose of this project is to obtain longevity mutants of _C. elegans_ and to characterize these mutants genetically, physiologically, and biochemically to understand the genetic basis of longevity and senescence. Dr. Mitchell and his colleagues have developed a technique by which mass quantities of age-synchronous nematodes could be obtained. This technique involves killing of eggs, by FUdr, present in culture with growing larvae and adults. They also attempted, unsuccessfully, to obtain x-ray resistant mutants. One of the interesting outcomes of these experiments was the observation that X-rays induced an increase in the life span of _C. elegans_. The three-day old adult organisms were exposed to 2,000 to 40,000 rads of X-rays. It was observed that such an exposure to X-rays increased the life span of _C. elegans_ by 3 to 5 days (10 to 20% increase in the life span.). No obvious defects in the appearance and in the function (production of eggs and oocytes, pharyngeal contraction, etc.) could be seen in these irradiated organisms. These observations on the radiation induced increase in longevity are compatible with the idea that the damage to DNA in the post mitotic cells could lead to a stimulation of the DNA repair system of these cells. Such an increase in the life span is probably not due to gene mutation. Life prolongation by nontoxic X-ray and U.V. irradiation has also been noted in insects, such as the flower beetle (Ducoff) and the protozoan Paramecium (Smith-Sonneborn).

Publications:

Mitchell, D.H., J.W. Stiles, J. Santelli and D.R. Sanadi. 1979. Synchronous growth and aging of C. elegans in the presence of fluorodeoxyuridine. J. Gerontology 34: 28-36.

Gandhi, D., J. Santelli, D.H. Mitchell, J.W. Stiles and D.R. Sanadi. 1979. A simple method for maintaining large, aging population of Caenorhabditis elegans. Mech. Aging and Devel. (In press).

Genetics and Biochemical Analysis of Aging. (P.I.: Dr. Michael R. Klass: R01-AG-01414-01).

The purpose of this study is to isolate and characterize mutants of *C. elegans* with an extended life-span, and to characterize biochemical aspects of aging processes in this organism. Dr. Klass exposed *C. elegans* to enthylmethanesulfonate, a mutagen, and then analyzed about 3,000 worms. Out of these, three "longevity mutants" were obtained. Unfortunately, these three mutant strains were not found suitable for further experimentation. The possibility cannot be excluded that the increase in the life span of these nematodes is due to mutation imposing dietary restriction rather than "longevity" gene mutation. Dr. Klass received this grant support in May 1979; he is continuing his search for longevity mutants of *C. elegans.* (Note, that BAP staff does not consider the molecular explanation of the "Mackey" effect to be trival at this stage of understanding in gerontology).

Age Related Alteration of Enzymes (P.I.: Dr. Morton Rothstein: R01-AG-00618-02).

Nematodes have been demonstrated as useful organisms to study age-associated alteration in enzyme function. Dr. Rothstein and his associates observed age-associated inactivation of the enzyme enolase in the nematode *Turbatrix aceti*. The rate of turnover of enolase is considerably decreased with age. The half-life of enolase increases from 58 hours in the young to 228 hours in the old worms. In contrast to the nematode, no, loss of activity of enolase with age was detected in rats. Dr. Rothstein's group further observed that the enzyme phosphoglucokinase of rat muscle cells is more unstable in young than in old rats. Dr. Rothstein proposes that the age-associated alteration in some enzymes is not due to errors in the protein sequence, but rather to a decreased protein turnover. How changes in protein turnover lead to enzyme activation remains unclear.

Publications:

Rothstein, M. and H.K. Sharma. 1978. Altered enzymes in the free-living nematode, Turbatrix aceti, aged in the absence of fluorodeoxyuridine. Mech. Age. Dev. 8: 175-180.

Sharma, H.K. and M. Rothstein. 1978. Serological evidence for the alteration of enolase in aging. Mech. Age. Dev. 8:341-354.

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Rothstein, M. 1979. The formation of altered enzymes in aging animals. Mech. Age. Dev. 9: 197-202.

Sharma, H.K., H.R. Prassana, R.S. Lane and M. Rothstein. 1978. The effect of age on enolase turnover in the free-living nematode, Turbatrix aceti. Arch. Biochem. Biophys. (In press).

The Molecular Basis of Neuromuscular Development (P.I.: Henry Epstein: R01-AG-1302-02).

The purpose of this project is to define structural and regulatory factors which control the assembly of myofilaments in C. elegans. The muscle activity is known to be impaired in the old nematode, C. elegans. Dr. Epstein is interested in determining if the loss of muscle function in C. elegans is due to the formation of defective muscle proteins, or to the way the muscle proteins are put together. His studies were done with various mutants of muscle functions. His results suggest that the aberrant muscle function is due to a defect in the structural organization of muscles, rather than in muscle proteins.

Publications:

Schachat, F.H., H.E. Harris, J.W. LaPointe and H.F. Epstein. 1977. Actin from the nematode Caenorhabditis elegans, is a single electrofocusing species. Biochem. Biophy. Acta 493: 304-309.

Schachat, F.H., H.E. Harris, R.L. Garcea, J.W. LaPointe, and H.F. Epstein. 1977. Studies of two body-wall myosins in wild-type and mutant nematodes. In. Eucaryotic Genetic Systems: ICN-UCLA Symposia on Molecular and Cellular Biology. VII: 373-380.

Epstein, H.F. 1979. Genes and muscles. Scientifc American. (In Press).

Some progress has also been made during the past year in projects in which other invertebrates, plants, and protozoa were used. These include:

Age-Associated Cellular Alterations in the Housefly (P.I.: Dr. Rajindar S. Sohal: R01-AG-00171-03).

Dr. Sohal has demonstrated that the rate of lipofuscin accumulation corresponds to the rate of aging in the house fly. He has found that the life-span of low-activity flies is more than twice as long as that of high-activity flies. The lipofuscin accumulates at a faster rate in short-lived, high-activity flies than in the long-lived, low-activity flies. The concentration of lipofuscin reaches the highest level in the long-lived flies at about twice the age of the short-lived flies. He interprets this as support of the view that lipofuscin is an indicator of physiological, rather than chronological, age.

In related studies he has demonstrated that, in general, the lysosomal enzyme activity in the housefly does not correspond to the mortality characteristics of the fly. This finding suggests that lysosomal enzyme activity may not be indicative of the physiological age of the organism.

Analysis of Development in Insects (P.I.: Dr. Judith H. Willis: R01-AG-00248-08).

The purpose of this study is to examine how juvenile hormone prevents passage through the developmental program exhibited by insects (silk moth, Cecropia) metamorphosis. The program has been defined by the changes in cuticular proteins secreted at each stage.

Dr. Willis and her associates have developed an _in vitro_ culture system in which diapausing moth pupal wing epidermis undergoes normal metamorphic development. Cuticular protein synthesis is initiated and sustained in tissue cultured for an extended period of time (5-17 days). This system, therefore, offers an opportunity to study changes in the pattern of protein synthesis which occurs during metamorphisis and during exposure of tissues to hormones. Dr. Willis' recent study suggests that in the presence of the molting hormone ecdysone, synthesis of 4 cuticular proteins is inhibited. Her group further demonstrated that the late final instar larval wing discs, in culture, give rise to pupal or adult structures depending on the ecdysone dose and the disc cell proliferation. The _in vitro_ systems developed by Dr. Willis and her associates would make it possible to study hormone action uncomplicated by endocrine feedback and degradation. This type of study could lead to the understanding of the developmental and aging processes of organisms.

Publications:

Walker, W.F. 1978. Mating behavior on _Oncopeltus fasciatus_ (Dallas): Effects of diet, photoperiod, juvenoids, and precocene II. _Physical. Entonal. 3:_ 147-155.

Nardi, J.B. and J.H. Willis. 1979. Control of cuticle formation by wing imaginal discs _in vitro_. _Devel. Biol._ (In Press).

Physiological Control of Differentiation in *Dictyostelium discoideum*
(P.I.: Dr. Barbara Wright; R01-AG-00260)

The overall objective of this research is to obtain data, *in vivo* and *in vitro*, essential to understanding metabolic transitions during differentiation, aging and death in a simple model system, *Dictyostelium discoideum*. Model systems such as *Dictyostelium* are highly amendable to analysis. Mechanisms operative in simple organisms also occur in more complex organisms.

Dr. Wright believes that the aging process must be understood in conjunction with an understanding of the differentiation process which preceeds it, and that this understanding will come most readily through an in-depth analysis of simple model systems such as *Dictyostelium discoideum*.

Glycogen phosphorylase is an enzyme of key importance to development and aging *Dictyostelium*. This enzyme catalyzes the conversion of cellular glycogen into precursors for the synthesis of new products (e.g., cellulose and trehalose) which accumulate during aging in this system. The mechanism by which the enzyme concentration changes during development were analyzed. Glycogen phosphorylase was purified to homogeneity and kinetically characterized. The purified enzyme was then used to elicit specific antisera in rabbits. This antisera, in conjunction with studies in which isotopes were incorporated into the enzyme, allowed an analysis of the changes in rate of enzyme synthesis and the rate of enzyme degradation during the aging process. The enzyme appeared *de novo* 5-6 hours after the initiation of differentiation, and changes in both its rate of synthesis and degradation could fully account for the change in concentration of enzyme protein.

It has been possible to alter the concentration of two critical saccharides in this system (trehalose and glycogen) about 6 fold by allowing development and aging to occur in the presence of exogenous glucose. Indeed, the increase in total carbohydrate in the cells could be completely accounted for by the measured increases in trehalose and glycogen. These "perturbation" studies, which are also being carried out with other exogenous metabolites, are interpreted with the aid of a computer model of this system. These studies also represent a powerful test of the model, which can be perturbed by a flux of glucose, to see whether alterations in metabolite levels in the model mimic those observed experimentally.

In connection with obtaining data for the kinetic model, four enzymes critical to metabolism in this system were further characterized: glucose-6-phosphate dependent glycogen synthetase, citrate synthase, glucokinase, and trehalose-6-P synthetase.

Publications:

Thomas, D.A. and Wright, B.E. 1976. Glycogen phosphorylase in *Dictyostelium discoideum*. I Purification and properties of the enzyme. *J. Biol. Chem. 251*: 1253-1257.

Thomas, D.A. Effects of adenosine 5'-monophosphate, glucose-1-phosphate and nucleotide sugars on the activity of glycogen phosphorylase. J. Gen. Microbiol. (in press).

Thomas, D.A. and Wright, B.E. 1976. Glycogen phosphorylase in *Dictyostelium discoideum*. II. Synthesis and degradation during differentiation. *J. Biol. Chem. 251*: 1258-1263.

Wright, B.E., Tai, A. and Killick, K.A. 1977. Fourth expansion and glucose perturbation of the *Dictyostelium* kinetic model. *Eur. J. Biochem. 74*: 217-225.

Saunders, D.A. and Wright, B.E. 1977. Characterization of glucose-6-phosphate-dependent glycogen synthetase from *Dictyostelium discoideum*. *J. Gen. Microbiol. 100*: 89-97.

Porter, J.S. and Wright, B.E. 1977. Partial purification and characterization of citrate synthase from *Dictyostelium discoideum*. *Archiv. Biochem. and Biophys. 181:* 155-163.

Killick, K.A. and Wright, B.E. 1978. Multiple forms of glucokinase from *Dictyostelium discoideum*. *J. Bacteriol.* 133: 1039-1041.

Killick, K.A. 1977. A radiometric assay for trehalose-6-phosphate synthetase. *Anal. Biochem. 79*: 310-318.

Killick, K.A. 1977. A coupled spectrophotometric assay for trehalose-6-phosphate synthetase from *Dictyostelium discoideum*. *Exptl. Mycol. 1:* 221-229.

Programmed Development and Senescence in Volvox. (P.I.: Dr. Gary Kochert; R01-AG-00127-01).

The goal of this project is to understand basic mechanisms of aging common to higher organisms. The specific aspect of aging under investigation in this research is programmed senescence. This theory postulates that the development and subsequent senescence of organisms is under the control of a sequentially expressed genetic program. Successive developmental stages would be characterized by the expression of different but overlapping batteries of genes. Once an organism reaches the last gene battery in its sequence, senescence ensues because specific "suicide genes" are turned on and/or by some other less direct means. Organisms or cells "escape" from senescence by some mechanism which enables them to return to the start of the genetic program. Unicellular organisms often present two fundamentally different cell types: somatic and reproductive. Somatic cells of multicellular organisms undergo senescence since they lose their ability to become reproductive cells again.

The concept of programmed senescence using Volvox carteri is being investigated. This organism has only two cell types: reproductive cells and only one kind of somatic cell. The organism has a short life cycle and can be grown synchronously so that large numbers of synchronously senescing cells can be obtained for biochemical analysis. One can also make genetic crosses; mutants have been found which the somatic cells do not senesce, but revert to reproductive cells and begin the genetic program anew. The organism thus have advantages for the study of aging which are not found in any other organism. Dr. Kochert hopes to learn more about basic mechanisms of aging by examining the program of development and senescence in this unusually favorable organism.

Newly formed Volvox colonies contain 12-16 reproductive cells surrounded by a single layer of 2,000-3,000 somatic cells. Each somatic cell is surrounded by a transparent sheath. The reproductive cells cleave to form new organisms. These resulting juvenile organisms are released by dissolution of a hole through the surrounding layer of somatic cells. The remaining somatic cells swim about for some time as a "hulk" or "ghost," but ultimately die. The duration of each phase of the life cycle has been measured under constant conditions. Somatic cells die and lyse about 30 hours after the juvenile colonies have been released. This is not affected if the reproductive cells are removed from the colony before they cleave. Senescence is thus intrinsic to the somatic cells. Production of sheath material is the most conspicuous biochemical activity of the sheath cells. Production of sheath causes the cells to move apart from one another and the colony to expand. By measurements on photographs, one can show the somatic cells of the parent apparently cease sheath production about the time of release of the juvenile colonies.

The sheath surrounding the somatic cells is removeable by proleolyic enzymes and stays in the supernatent on subsequent centrifugation. One can use this technique to measure incorporation of radioactive precursors into the sheath.

When measured by this method sheath production is also seen to stop about the time of juvenile colony release.

Methods of large scale separation of the somatic cells of the parent colony at all developmental stages have been developed. Total colony protein and total somatic cell protein has been measured at several developmental stages. Total colony protein show a peak during early developmental cycle, but total somatic cell protein show a peak during early development and subsequently declines as senescence occurs. The pattern of protein synthesis during development and senescence have been examined by one dimensional SDS electrophoresis and by two dimensional electrophoresis using isoelectric focusing in the first dimension and SDS electrophoresis in the second dimension. With the latter technique, large numbers of proteins can be resolved. Clear differences between the proteins made by somatic and reproductive cells can be seen. Moreover, one can observe the somatic cells change from one battery to another during development. At the latest stage investigated, (a hulk stage), only a few proteins are still being made, all a subset of the lost gene battery. This change in gene battery occurs with no apparent change in the external morphology of the somatic cell.

Publications:

Gale, J.G. 1978. Production of extracellular sheath in Volvox. M.S. Thesis, University of Georgia.

Hagen, G. Protein synthesis during Volvox senescence. Ph.D. Dissertation, University of Georgia (In press).

Aging and Temporal Patterning in Tetrahymena. (P.I.: Dr. David Nanney; R01-AG-00010).

Clones of ciliated protozoa change in a systematic manner as the time since fertilization increases. Immediately after conjugation, clones of Tetrahymena are immature, and are unable to mate again until a strain-specific number of cell divisions has occurred. The number of cell divisions required may be modified by manipulations of the environment, but is remarkably constant for a particular growth regimen. Under standard conditions in T. thermophila, maturation requires 40-60 cell divisions. Another temporal pattern concerns the expression of allelic traits: Antigens or isozymes or morphotypes. In each case a young clone characteristically manifests both specificities with co-dominant allels or only the dominant trait for fully dominant alleles. In most cases T. thermophila, however, subclones are eventually produced which express one or the other allele, regardless of the dominance relations. This process is referred to as allelic assortment, and it may begin at different times for different loci. Different loci may also be specific in the relative numbers of subclones of the alternative types; some loci give equal outputs and some give biased ratios. A third time-related process concerns breeding performance. When clones first become mature most of the progeny produced at conjugation survive, but as the parental clones become older, the probability of survival progressively declines; clones a few hundred cell divisions old (or a few years old) have, on the average, a much reduced probability of surviving conjugation. The three classes of temporal program concern three different cellular properties, a) the physiological state of the macronucleus, b) the genetic composition of the macronucleus, and c) the genetic integrity of the micronucleus. We hope to discover the mechanisms regulating the changes.

The most recent studies on the control of maturation in tetrahymena have been concerned with environmental effects on the time of sexual maturity, and particularly with the phenomenon of "early maturity". Previous studies in the laboratory have shown that mutations at several loci can reduce the immaturity period from about 50 to about 15 cell division. All early maturity mutants, moreover, show slow and erratic growth following conjugation. The newer observations indicate that suboptimal environmental conditions can also induce early maturity during a critical period following fertilization. Either high temperature shocks or high salt (calcium chloride) concentrations are effective. The results suggests a general early maturity shunt, triggered by a genetic-environmental incompatibility. The mechanism is not understood.

The studies on allelic assortment are now focused on the effects of varying gene dosage on the assortment patterns. Particularly, we have learned to construct clones with haploid and triploid nuclear primordia and from these we have secondarily developed specific aneuploid strains with particular conbinations of genetic markers. Assortment patterns in these special strains seem to require a macronuclear organization in which the replicons are associated into supra-chromosomal arrays.

Studies on germinal decay consist thus far of retrospective surveys directed to the question of rates of loss of micronuclear capabilities. Generally, the onset of sterility appears to be irregular, with some lines quickly becoming defective and other persisting for several years without appreciable decline. The results are consistent with a mutational basis, and a rate of loss of micronuclear function of about 8-10% per year in many inbred stocks, but much higher in certain strains.

Publications:

Nanney, D.L. 1977. Traumatic induction of early maturity in Tetrahymena. Genetics 86: 103-112.

Preparata, R.M. and Nanney, D.L. 1977. Cytogenetics of triplet conjugation in Tetrahymena: origin of haploid and triploid clones. Chromosoma 60: 49-57.

Simon, E.M. and Nanney, D.L. 1979. Germinal aging in Tetrahymena thermophila. Mech. of Aging and Devel. (to be submitted).

THEORETICAL GERONTOLOGY, BAP

Overview: NIA extramural research involving simulation and modeling, and research on population genetics are supported in this BAP subprogram of Theoretical Gerontology. Simulation studies are encouraged both as data-driven and as theoretical models. Data-driven studies are those which the modeling and computer simulation are dependent upon experimental data for development. Experiments, for example, can be designed to quantify all interdependent metabolites and metobolic processes within the scope of the simulation model. The model integrates these data, and analyzes relationships based on unknown or hypothetical pathways and interdependences.

The simulation models becomes valuable when sufficiently sophisticated to predict functions describable as experimentally testable hypotheses. Data driven simulation research is encouraged at the molecular/biochemical, physiological, and cellular (cell-lineage) levels. The basis for support of such studies is the probable need for integration of many variables to achieve knowledge of mechanisms of senescence or longevity. This concept recognizes the extreme complexity of an organism, and the likelihood that single-variable, cause and effect experimentation may perturb the rate of aging in an organism, but less likely explain underlying aging mechanisms.

The abstract modeling enables elaboration of mathematical concepts of heuristic value to gerontology. This type of simulation can provide a guide to gerontological theory development, however its usefulness ultimately lies in an ability of the models to predict biological functions testable in the laboratory. Although hypotheses testable on laboratory animals are the goal of NIA-supported simulation research, the abstract simulation may contribute most directly to the development of concepts and computer models for data-driven simulation.

Knowledge of the fate of individual cells and their progeny is of paramount importance to the understanding of senescence. In the organism this information is sought from the zygote through embryogenesis, development, and eventually through cell attrition and tissue involution that appears related to senescence. Similarly, in clones of protozoa, and populations of cells in culture, a knowledge of cell lineages is essential to the design of experiments addressing senescent changes at the cellular level. Simple eutelic metazoans, such as nematode Caenorhabditis, offer promise of complete analysis of all cell lineages. This will enable determination of any age-associated cell loss, and enable prediction of the role and mechanism of such loss. In large organisms, and those in which development is less deterministic, a more generalized lineage analysis is achievable, and has similar value in predicting the role of senescent changes in populations of cells.

Population genetics is a field which also relies, in large measure on modeling and computer simulation to integrate the complexities of genetic composition, rates of change and phenotypic expression in a population of organisms. In the context of NIA program needs, population genetics encompasses qualitative genetics. To gerontology, this field of genetics offers insight into the evolution and maintenance of the genetic component of senescence, longevity, and within species and between sexes the relative contributions to longevity of the environment and genetic constitution of an organism. Research in these aspects of population genetics is encouraged from lower organisms through man. Within Theoretical Gerontology there are supported 2 research grants.

Accomplishments: This subprogram area supports only two projects on data-driven simulation at the laboratory of Dr. Barbara Wright in Boston. Dr. Wright and her associates have made significant progress in this area. Some of their findings are summarized below.

Computer Analysis of Differentiation (P.I.: Dr. Barbara Wright; R01-AG-00433-10).

The overall objective of this project is to simulate all of the sequential biochemical events leading to differentiation and aging in the slime mold *Dictyostelium discoidium*. Various metabolic complexities can be analyzed adequately in this way, and this could lead to the understanding of critical variable underlying differentiation and aging.

Dr. Wright exposed the slime mold, *D. discoideum*, to exogenous glucose, uracil, and inorganic phosphate for 90 and 900 min. to determine their effect on the cellular levels of glucose-6-phosphate, uridine-diphophate-glucose, glycogen, trehalose, and cellulose. External fluxes of these metabolites were also imposed on the kinetic model of carbohydrate she has constructed, to achieve simulation of the experimental results. One of the major discrepancies found between the experimental model and the simulation studies was the concentration of glucose-6-phosphate. This discrepancy predicted compartmentation of glucose-6-phosphate. Experimental evidence has now been obtained to confirm this prediction. Dr. Wright is in the process of constructing a steady-state model of flux through the citric acid cycle and pentose metabolism.

Publications:

Kelleher, J.K., and P.J. Kelly, and B.E. Wright. 1978. A Kinetic Analysis of glucokinase and glucose-6-phosphate in *Dictyostelium*. *Mol. Cell. Biochem. 19:* 67-73.

Killick, K.A. and B.E. Wright. 1978. Multiple forms of glucokinase from *Dictysotelium discoideum*. *J. Bacteriol 13:* 1039-1041.

Overview: The mechanisms responsible for age-associated changes in skin are unknown. Even the basic descriptive biology of the changes associated with skin aging remain inadequately studied, as the necessary research efforts have been wanting. In contrast to this lack of research, the pressing need for a knowledge base from which to address skin aging problems is highly visible. The age-associated changes in skin cause adults, initially, modest physical discomfort, and very marked concern associated with self-image and appearance-based age stereotyping. As a person becomes older, the concerns for the appearance of skin become more than matched by serious discomforts such as chronic dryness and itching. Many of the remedies people seek to ameliorate problems, both discomfort and appearance, do not have a sound basis in research derived knowledge and not only may be of questionable value, but may aggravate an existing skin problem. A concerted research program on skin aging will provide the knowledge base for interceding with environmentally induced skin aging, and perhaps even modify the rate of intrinsic processes. As complex as skin is, it none-the-less appears to be one of the most accessible, and in relative terms, simplest of organs. The molecular and cell biology of aging skin serves, therefore, a secondary function as a model that may prove instructive of aging processes in tissues and organs where the human consequences of the expression of senescence is much more severe.

Research on skin will be pursued at the basic biological level to understand the mechanisms of skin aging, and at the clinical level including fundamental studies necessary to better define the nature and extent of the skin problems with which the elderly are confronted. There is no clear interface between the biomedical molecular and cellular research and the clinical research supported in this subprogram. At the basic end of the spectrum, in vitro skin cell biology will be pursued in the subprogram of Genetics and Cellular Aging. The cell culture model is probably most fully developed for skin-derived cells, not only fibroblast-like cells, but also keratincytes. Pursuit of the relationship between data derived from cell-culture models, and that derived from intact skin, may provide the first understanding for rational interpretation of cell-culture findings. Studies on skin will be conducted to determine the range and adequacy of the care and treatment for skin problems. Medical studies will include cell kinetics in the dermis and epidermis; change in microvascular, elastin, collagen, mucopoly-saccharides, the basal lamina, pigmentation, keratinization, hair follicles, sebaceous secretion, apocrine glands, sweat glands, and nails. Changes in the capacity of skin to adapt to modifications in surface area, as following weight loss, requires study. Similarly, the basis for skin sagging, and apparent increase in surface area without change in body mass should be investigated. Finally, the aging of skin may reduce its effectiveness as a barrier to environmental insult, including protection from temperature extremes.

Skin, the most accessible of organs, is probably the organ most likely to yield the earliest of fundamental knowledge of the aging process in man. It would follow that clinical advances are equally promising. Within

It would follow that clinical advances are equally promising. Within Dermatology there are supported 6 research grants.

Accomplishments: Considerable progress has been made during the past year in a program project entitled: "Model system to study accelerated cutaneous aging." (P.I.: Dr. D. Martin Carter; P01-AG-01012-02). This program project is composed of three projects, one of which (Carter) is identified with the BAP subprogram, Genetics and Cellular Aging. The accomplisments of each is described, briefly below:

Cellular Senescence After DNA Damage: Dr. D.M. Carter

The purpose of this project is to study: (1) growth patterns of cultured cutaneous cells obtained from normal individuals of different ages and from persons exhibiting evidence for accelerated aging because of heritable diseases (e.g. dyskeratosis congenita) or because of environmental exposure, such as PUVA (psoralen and UV-A treatment for psoriasis) treatment; (2) repair of psoralen-DNA photoadducts produced in vitro; (3) cytogenetic phenomena related to exposure to psoralen plus light; and (4) blood concentrations of psoralens in therapeutic range.

Dr. Carter and his associates have established, during a short period of time, cultures from skins of normal persons and patients suffering from a variety of skin diseases such as psoriasis, dyskeratosis, congenita, lipoid proteinosis, acne keloidalis, cowden syndrome, leg ulcers, and multiple malignancies.

Dr. Carter's group has observed an increase in the population doubling times in cultures exposed to increasing concentrations of psoralen + UV-A. This treatment also leads to a decrease in cell density and H3-thymidine labeling index. These results were obtained from fibroblast derived from cutaneous and extracutaneous sites from donors of different ages. PUVA treatment seems to induce a G2 block in the cell cycle.

Studies on the repair of psoralen-DNA cross-links suggest that such products disappear within 24 hours of treatment in fibroblast of normal persons of various ages. In contrasts, the psoralen cross-link removal is slow in individuals suffering from dyskeratosis congenita. Psoralen seems to increase sister chromatid exchange in peripheral blood leukocytes. Dr. Carter and his associate have refined their technique for detection of psoralen in the blood or plasma in nanogram quantities. This refinement of the technique for psoralen detection should enable quantitation of biologic effects of PUVA damage and its repair.

Ultrastructural studies of the responses of microcirculation, dermal elements and epidermis to PUVA therapy: Dr Irwin Braverman

Dr. Braverman has compared the ultrastructure of the sun-exposed forearm skin and sun-protected buttock skin of six individuals 23 to 75 years old. Samples of buttock skin of 30 patients, before or after PUVA treatment (for various times), have been examined. The sun-exposed forearm skin exhibited and increase in collagen in the papillary dermis. The collagen fibrils are normal in appearance, but densely-packed and often disorganized in orientation within collagen buddles. The structure and density of elastic fibers in between and below collagen bundles appear abnormal in the papillary dermis. The reticular epidermis as well as epidermal cells and melanocytes appear normal under EM.

Vascular wall thickening also increased in the papillary dermis of the sun-exposed forearm skin. Both venules and arterioles were affected. The increase in the vascular wall is due to deposition of homogeneous amorphous basement membrane-like materials around the periphery of the wall. Such materials are probably laid down by veil cells (fibroblasts) that surround the microciculatory vessel. Identical changes were seen in the cutaneous vessels of patients with diabetes mellitus.

Priliminary study of the buttock skin of PUVA patients shows identical collagen, elastic fiber, and vascular changes to those seen in the forearm sun-exposed skin of control individuals. PUVA does cause elastosis, and the elastosis change may be related to age as well as ultraviolte damage.

Functional and molecular markers of aging in epidermis: Dr. Joseph McGuire

The purpose of this project is to study the structural proteins of keratinocytes, and functional markers for keratinocyte differentiation. During the first year of this project, techniques for isolation and characterization of vaious keratins from calf hoof have been developed. Such techniques will be applied for human keratins. Dr. McGuire's group has already been able to isolate specific keratins from scales produced in various skin disorders. These keratins have been labeled (by formulation), separated and identified.

Publications:

Cassuto, E., D.M. Carter. 1979. Interstrand cross-links in DNA of phase lambda after exposure to 8-methoxypsoralen and trimethylpsoralen in the presence of light. J. Invest. Dermatol. 73:00-000. (In press).

Carter, D.M. and S.R. Cohen. 1979. Prenatal-diagnosis of heritable skin diseases In Dermatology Update: Reviews of Physicians. S.L. Moschella, Ed., Elsevier North Holland, New York. (In Press).

Carter, D.M., M. Pan, and J. Varga. 1979. Pigment response of melanoma cells to psoralens and light, Proceedings of the Xth International Pigment Cell Congress, Karger, Basel. (In press).

Carter, D.M., M.S. Pan, A. Gaynor, J.S. McGuire and L. Sibrack. 1979. Psoralen-DNA cross-linking photoadducts in dyskeratosis cogenita: Delay in excision and promotion of sister chromatid exchange. J. Invest. Dermatol. 73: 00-000. (In press).

Carter, D.M., A. Gaynor, and J.S. McGuire. 1978. Sister chromatid exchanges in dyskeratosis congenita after exposure to trimethyl psoralen and UV light. In: DNA Repair Mechanisms, (ICN-UCLA symposia on Mol. & Cell. Biol., Vol. 9) eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox.

Academic Press, N.Y. pp. 671.-674.

Milstone, L.M. and J.S. McGuire. 1978. Comparison of proteins that form 8-10nm filaments in two keratinizing epithelia and endothelial cells in culture. J.Cell. Biol. 79: 30a.

DiPasquale, A., J.M. Varga, G. Moellmann and J. McGuire. 1978. Synthesis of a hormonally active conjugate of alpha-MSH, Ferritin and fluorescein. Antalytical Biochemistry 84: 37-48.

Milstone, L.M. and J. McGuire. Homologies in the keratin polypeptides of calf hoof, J. Invest. Dermatol. (In press).

Sibrack, L.A. and I.H. Gouterman. 1979. Cutaneous manifestiations of diabetes mellitus. Cutis, November. (In press).

Scollay, D. and L.A. Sibrack. The systemic manifestation of generalized exfoliative dermatisis. In: Dermatological Signs of Internal Disorders. ed. J.P. Collen, Yearbook Medical Publishers. (In press).

GENETIC AND CELLULAR RESOURCES, BAP

Overview: Fundamental to gerontological research is the availability of high quality and well-characterized biologics. The Genetic and Cellular Resources component of BAP is responsible for biologics supply, characterization, and related services in the fields of cell culture, invertebrates, plants, prokaryotes and, in collaboration with the Animal Resource Unit of BPAP, chimeric and genetic mosaic animals. These services are in support of both BRCM-supported research and research ongoing within the NIA Intramural Research Program. Research in this subprogram supported by grants includes studies on cell lineages and population characteristics of human diploid cells in culture; the establishment, isolation, and characterization of longevity-mutant nematodes, and the research training of post-doctoral scientists in the technologies of tissue-specific cell line development. Resources supported by contracts are: The NIA Cell Line Respository, which supports acquisition, characterization, and distribution of cell lines of special utility to NIA grantees, prospective grantees, and other gerontologists; and the Mycoplasma Contamination Testing Service, contributing to quality control in cell culture laboratories for the same group of investigators. Detailed information on these contracts has been published in two articles: "National Institute on Aging Cell-Line Repository." 1978, *Exp. Aging Res. 4*: 321-331. (N.K. Das and D.G. Murphy) and "National Institute on Aging *Mycoplasma* Testing Service", 1978. *Exp. Aging Res. 4*: 333-341 (N.K. Das, D.G. Murphy and I.J. Fred).

NIA is in the process of awarding another contract, "*Caenorhabditis* Genetic Center" (CGC). This is to be a *C. elegans* stock center with responsibilities of acquisition, banking and distribution, and related services, as maintenance and distribution of the genetic map. It is planned to meet the existing and future needs of the community of scientists conducting research with C. elegans including, but not limited to, research identified with the cagegorical responsiblities of the NIA. Advice on pertinent science policy and practice for the CGC has been, and will continue to be sought from qualified *ad* hoc advisors to the NIA. Within Genetics and Cellular Resources there are supported 8 research grants.

Accomplishments: This subprogram includes research on cell lineage and population characterisitss of human diploid cells in culture. The progress of this project is summerized below.

Analysis of Aging in Cell Lineage. (P.I.: Dr. Euguene Bell; P01-AG-00354)

In this project, Dr. Eugene Bell and his associates at MIT are studying human foreskin fibroblast-like cells in culture to determine how individual cells contribute to the overall decline or "aging" of the mass cultures. Cell growth patterns were filmed for several weeks and the filmed data were analyzed by computer. Results show that) the nondividing, phase III cells are descendants of different cell lineages with varying duration of generation time and proliferative capacity, b) cells at *all* population doubling levels exhibit heterogeneity with respect to cell size and motility, and, c) the phase III cells are not senescencing cells, they can be kept alive for an additional 10-12 months in an appropriate culture medium. Based on these results, Dr. Bell and his colleagues postulate that the loss of *in vitro* proliferative ability of cells is an indication of cell differentiation rather than cellular aging (Bell *et. al.* 1978). This study raises some questions on the validity of the use of *in vitro* fibroblast-like cell culture system as a model for cellular aging. However, further studies are necessary on cell lineages, the functional ability of non-proliferating fibroblast-like cells, and the relationship, if any, of the *in vitro* cells at various stages to normal *in vivo* cells.

Bell, E. L.F. Marek, D.S. Levinstone, C.Merill, S. Sher, L.T. Young, and M. Eden. 1978. Loss of dividion Potential: aging or differentiation. *Science* *202*: 1158-1163.

The BAP subprogram on Genetics and Cellular Resources supports two contracts, and is in the process of awarding a third. The activities of these contracts are summarized on the following pages.

Contract Number: N01-AG-2865

Contract Title: Selection, Production, Characterization and Distribution of Gentically Marked Cells for Aging Research

Contractor: Institute of Medical Research, Camden, New Jersey
Principal Investigator: Dr. Warren W. Nichols

Money Allocated: $178,657

Objectives:

1. Provide standard, highly-characterized, extensively banked lines of normal human diploid fibroblast-like cells.

2. Provide banked and characterized, genetically marked or mutant cells identified as possessing features of value in probing mechanisms of cellular aging.

3. Provide standard, characterized, moderately banked lines of tissue-specific cell types.

4. Contribute to the development of the field of cellular aging theory, concepts, and techniques, through consultation and workshops, particularly in the areas of somatic cell genetics and cytogentics.

5. Limited cytogenetic screening for aging research.

Significance to aging Research:

Events at the cellular level are probably major determinants of the expression of longevity and senescence at the level of the organism. The techniques of cell-culture enable investigation of cellular events consequent to aging independent of the complexity of the whole organism. Such *in vitro* studies are conducted on cells as they age in the cell culture enviornment, and also, on cells derived from people and experimental animals of different ages. Most such studies to-date are on fibroblast-like cells. Advances in cell-culture technologies show considerable promise that an increasing number of tissue-specific cell types, that is differentiated cells in culture, will become available to the gerontologist for comparative studies of cellular aging. The contractor is to provide leadership in the acquisition, banking and distribution of differentiated cell lines valuable to the gerontologists. The contracted resource also supports NIA interest in knowledge of genetic mechanism of cellular aging approached through the use of genetically marked cell-lines and use of the techniques of somatic-cell genetics.

Contract Progress

The NIA Cell-Line Repository has established 236 cell lines. These include the female and male fetal lung fibroblasts (IMR-90 and IMR-91); fibroblasts obtained from skin biopsies of normal individiuals of various ages, as well as newborn and fetal materials; fibroblasts from individuals with premature aging syndromes, such as progeria, Werners syndrome, Alzheimer's disease and Mulibry nanism syndrome; fibroblasts from the chromosome breakage syndromes and abnormal DNA repair such as Bloom's Syndrome, ataxsia telagiectasia, Fanconi's anemia and *Xeroderma pigmentosum*. Cells that maintain a differentiated function in culture are also banked and these include endothelial smooth muscle, renal epithelium and endometrium cells. In this latter category both human and animal cells are included. The temperature sensitive, cell-cycle animal mutant cells are included in the repository. All frozen cells are prescreened for *Mycoplasma*, bacteria, yeast, and mold contamination. Karyotyping and selected isozyme studies and species identification are also carried out, as necessary.

The two highly characterized cell lines are IMR-90 and IMR-91. The characterization studies include chromosome banding, frequencies of chromosomal abnormalities during culture lifespan, cell kinetics, sister-chromatid exchanges during culture lifespan, isozyme analysis, HLA typing and a variety of growth parameters. These cell lines have been developed and stored in large quantity at early passage and at intervals throughout their life span with eventual aim of replacing the existing stock of WI-38 cell-line as it is exhausted. The IMR-90 cell has found extensive usage not only in gerontology, but by the biomedical research community, in general. During the past year there have been 202 requests for IMR-90 starter cultures, compared to 178 requests for other cell lines.

There have been considerable interest in the possibility of using IMR-90 and IMR-91 as substrates for vaccine production. Two commercial companies and the U.S. Army have been making trial vaccines with IMR-90. Feedback from these trials has indicated that the cells are satisfactory for this use. Bureau of Biologics, NIH, is also interested in IMR-91 as a vaccine substrate. The possibility of obtaining a patent on IMR-90 or IMR-91 for vaccine production was discussed with the Bureau of Biologics, but this action seems unlikely.

The NIA Cell-Line Repository has achieved significant contribution to the cellular aging research community, a contribution expected to increase as the diversity of cell types in culture expands.

Publications:

Nichols, W.W., D.G. Murphy, V.J. Cristofalo, L.H. Toji, A.E. Green and S.A. Dwight. 1977. Characterizaton of a New Human Diploid Cell Strain, IMR-90. Science 196: 60-63.

McGarrity, G., D. Murphy and W. Nichols (eds.). 1978. Mycoplasma Infection of Cell Cultures. New York: Plenum Press.

Nichols, W.W., R.C. Miller and C. Bradt. 1977. In: Handbook of Mutagenictiy Test Procedures. B.J. Kilbey, M. Legator, W.W. Nichols C. Ramel (eds.). pp. 225-233. Tesevier Scientific Publicaton Co.

Nichols, W.W. and D.G. Murphy (eds.). 1978. DNA Repair Processes and Cellular Senescence. Symposium Specialists, Miami, Florida.

Nichols, W.W. and D.G. Murphy (eds). 1979. The Use of Differentiated Cells in Culture for Biomedical Research. Internat. Rev. Cytol. (in press).

Contract Number: N01-AG-8-2117

Contract Title: National Institute on Aging Mycoplasma Contamination Testing Service

Contractor: Institute for Medical Research, Camden, New Jersey
Principal Investigator: Dr. Gerard J. McGarrity

Money Allocated: $47,572

Objectives:

1. The detection, prevention, and control of Mycoplasma infection as a service, primarily to NIA grantees and the NIA Intramural Program.

2. Provide consultaiton to laboratories having chromic problems with mycoplasmas and other micro-organisms.

3. Distribute a quarterly newsletter on quality control of cell cultures.

Significance to Aging Research:

The NIA encourages the use of cell-culture technologies to pursue knowledge of genetic, molecular and cellular mechanisms of human aging. Investigators working with cultured cells must exercise extreme caution to avoid microbial contamination of those cultures. Gross mibrobial infections due to bacteria or fungus usually are obvious and rapidly destroy the contaminated culture. It is the more subtle infections, as with Mycoplasma, which may go undetected. Mycoplasmas, the smallest known free-living organisms, are common contaminants of cultured cells. They are difficult to detect, and while their presence may not destroy the host cell-culture, it often alters the metabolism and function of the cultured cells. Unlike bacteria, mycoplasma lack cell walls and are resistant to many common antibiotics. Mycoplasma contamination is of particular concern in gerontological research in which it is often necessary to maintain cell-cultures for a relatively long period of time.

Contract Progress

During the first year of this contract a total of 501 cell cultures were submitted to the NIA Mycoplasma Testing Service from 19 laboratories. A total of 42 laboratories, (GRC, NIA grantees and contractors) were elegible to submit cultures during the first 12 months of this contract. Of the 501 cultures submitted, 482 cultures were actually assayed for mycoplasmas. The remaining 19 were found to be either grossly contaminated by bacteria, yeast or mold, or had large visible damage to the cell culture flask. Mycoplasmas were detected in 40 of the 482 cultures assayed, and annual infection rate of 8.3%. Table A lists the number of cultures tested and the mycoplasma isolation figures for each quarter.

Organisms isolated from positive cell cultures included A. laidlawii, M. hyorhinis, M. orale and M. arginini. When infected cultures were detected, the participating laboratory submitted an increased number of cell cultures for assay to determine the extent of mycoplasmal infection in that laboratory. Several laboratories have also contacted the principal investigator for in depth consultation regarding methods of control and prevention.

The circulation of the informal communication "Tissue Culture Quality Control" increased in each of the first three quarters. This is expected to continue. Thirty were distributed to submitting laboratories in the first quarter. This number was increased more than 5 fold during the next two quarters without any publicity. During the fourth quarter, announcements of the availability of the communication were mailed to ASM News of the American Society of Microbiology and TCA Report of the Tissue Culture Association. This should increase awareness of the objectives of this program and should improve standardization for cell cultures in all facilities.

The service requires rigid internal quality control and standardization to maximize efficiency of detection methods. Potential difficulties were encountered when the source of yeast extract for all commercial suppliers was changed. Yeast extract is a component of mycoplasma media. Quality control studies enabled the contractor to select a source of yeast extract that will promote the growth of cell culture mycoplasmas. Similar studies showed that substitution of mycoplasma broth base resulted in better growth of an uncloned isolate of M. hyorhinis than the previously used brain-heart infusion.

Several publications authored or co-authored by the principal investigator have been published during the first contract year. Several invited presentations relevant to this service have also been made.

It is believed that the objectives for the first year of this contract have been fulfilled. This testing program has increased the awareness of NIA grantees and contractors to the problems of mycoplasma infection and other aspects of quality control. The detection of mycoplasmas in 40 cell cultures that were previously used in NIA financed studies signifies the high cost-effective nature of this service. The elimination of these infected cultures from the submitting laboratories should also result in improved standardization of experimental studies. The testing service will see greater utilization and greater impact during the second contact year.

Publications:

McGarrity, G.J., V.Vanaman and J. Sarama. 1978. Comparative studies to detect mycoplasmas in cell cultures by uptake of radiolabelled uridine/uracil and by culture. Zentr. f. Bakteriol. Para. Infektion and Hyg. 241:252.

Diggelon, O. Vane, S. Shin and G.J. McGarrity. 1978. Endogenous HPRT activity in mycoplasmas isolated from cell cultures. *In Vitro* 14:734-739.

McGarrity, G., J. Sarama and V. Vanaman. 1979. In: *Methods in Enzymology* (eds. W.B. Jakoby and I.H. Pastan). Academic Press, N.Y., pp. 439-444.

McGarrity, G.J., V. Vanaman and J. Sarama. Detection of cell culture mycoplasmas: Comparative studies using uptake of radiolabelled uridine and uracil and microbiological culture. Exp. Cell Res., (in press).

Table A. Mycoplasma Infection of Cell Cultures by Quarter

	1978				1979	Totals
	1	2	3	4	1	
No. of cultures Submitted	-	89	139	83	190	501
No. of cultures Tested	-	86	135	78	184	482
No. of cultures Infected	-	7	2	20	11	40

Contract Number:

Contract Title:

Contractor: (To be determined)

Money Allocated:

Objectives:

1. Acquire, maintain and distribute wild-type and mutant strains of *C. elegans* for biomedical research.
2. Determine that the nematodes for distribution are free from contamination and conform to visible phenotype description.
3. Conduct yearly survival tests on a limited number of frozen strains, and test potentially advantageous new methods for strain storage and distribution.
4. Maintain and distribute the genetic map, nomenclative informaion and related data.
5. Identify mutant strains of special significance to aging research.

Significance to Aging Research:

This resource is in support of all scientists qualified to study and/or are training to study, *Caenorhabditis* elegans as a model organism in biomedical research. This policy appreciates that the entire range of research being conducted on *C. elegans*, mostly very fundamental molecular genetics and developmental biology, is of immeasurable value to gerontology. It is this same community from which the NIA seeks to stimulate grant applications in aging based on an existing foundation in embryology, growth and development.

The *Caenorhabditis* Genetic Center (CGC) is of special significance to gerontologists. *C. elegans* manifests typical life span phenomena of species-specific longevity, post-reproductive onset of senescence, followed by morbidity and death. It is a simple organism and it has a short lifespan. It is rapidly becoming genetically characterized, and is cultured with relative ease. C. elegans offeres promise of being among the organisms offering the earliest insights into mechanisms of longevity and senescence. The CGC should greatly facilitate acquisition of this knowledge.

The objectives of the Immunology Program are to understand: (1) the fundamental molecular and cellular changes responsible for the decline in immune competence with age; (2) the relationship between immune dysfunction and age-related pathologies at the molecular, cellular, and organismic levels using human and animal models; (3) the development of replacement therapies and other treatment modalities to augment immune function; and (4) the application of new knowledge to clinically relevant situations involving elderly subjects. This program currently supports 42 research and training grants, fellowships, and contracts. The total FY 78 activity was $2,400,000 and thus far the total activity for FY 79 is $3,100,000.

The objective of the Pharmacology Program is to develop new knowledge related to basic and clinical expressions of age changes which affect the utilization of drugs. General areas of investigation include: (1) distribution, metabolism, and pharmacokinetics of therapeutic drugs in older subjects as they may differ from other age groups; (2) drug interactions in aged patients receiving multiple drugs; (3) paradoxical reactions to drugs; and (4) environmental factors affecting drug efficacy or toxicity. Particular attention is given to studies of drugs most frequently prescribed for older age groups. The program currently supports 15 research and training grants. The total FY 78 activity was $420,000 and thus far the total activity for FY 79 is $1,160,000.

The Intermediary Metabolism Program supports research concerned primarily with investigations of age-associated changes at the subcellular level. This includes studies which examine the changes in subcellular organelles and their components which occur with aging, as well as studies which examine age-associated changes in individual molecules within a cell. The program currently supports 34 research and training grants and fellowships. The total FY 78 activity was $2,775,000 and thus far the total activity for FY 79 is $3,400,000.

The Diabetes Program supports research concerned with the diagnosis, treatment, and prevention of diabetes in the elderly. The scope of the program is broad and includes basic biomedical, clinical, and behavioral areas of research. The program currently supports 6 research grants and fellowships. The total FY 78 activity was $590,000 and thus far the total activity for FY 79 is $800,000.

The four major sub-program areas - Immunology, Pharmacology, Intermediary Metabolism and Diabetes and Aging - have witnessed an increase in public interest as judged from the oral and written inquiries, number of applications, and scientific merit of those applications reviewed by the various DRG Study Sections.

The Immunology Program sponsored a major international workshop on "Immunological Aspects" of Aging in May 1979 and recently issued a program announcement on Immunology in the NIH Guide for Grants and Contracts. These activities and others anticipated, will hopefully increase the number of applications and expand the scope of research activities. Requests for support and the quality of applications in the Pharmacology Program have increased due in part to the program announcement which appeared at the end of FY 78 (September 1, 1978). Also in FY 79, a program announcement on the psychosocial problems of Diabetes was issued jointly with NIAMDD, NICHD, NHLBI, and NIMH. NIA provided partial support for an NIH Conference on "Psychosocial Factors in Diabetes" as a follow-up to the issuance of the program announcement.

The Molecular and Biochemical Aging Program (MBAP) recognizes deficiencies in clinical research, therefore, several initiatives will be addressed in future program announcements and workshops/ conferences. In Immunology, MBAP will seek to understand more about the relationship between aging, impaired cell-mediated immunity and impaired resistance to bacterial and viral infections. Also, an effort will be made to encourage research and training on the possible immunological basis of Alzheimer's Disease. In Pharmacology, a specific effort will be made to increase the opportunity for training physicians in disciplines related to clinical pharmacology and pharmacokinetics. Because Diabetes in either its insulin dependent form or its non-insulin dependent, glucose intolerant form constitutes a major health problem for the elderly, an effort will be made to encourage investigations related to clinical manifestations. Specific attention will be given to the detection and diagnosis of diabetes in the elderly.

The conduct of research over the lifespan of animal models necessitates the provision of sufficient numbers of aged animals to permit investigators an opportunity to do pilot studies. It is therefore essential that NIA maintain animals of appropriate genetic background and age. The current resource is barely adequate to meet the demand. The colony must be improved in both numbers and specific strains to meet future needs.

Major Developments

Immunology

Since immunologically incompetent individuals are very susceptible to infections, immune dysfunction is probably one of the major sources of health problems of the elderly. The pathogenesis of a variety of age-related diseases may have an immunological basis. The overall aim of the research is to define the immunologic impairment in aging animal models and man.

Current studies are related to: (1) the basic mechanisms of immune cellular senescence; (2) the influence of genetics on immune expression; (3) the mechanisms of immunoregulation and immunodeficiency; and (4) the immunological basis of various pathologies.

Specifically, research is underway on a variety of topics including: the possible relation of aging to the effects of long term autoimmune reactions arising in the body; the nature of impaired humoral immunity in mice; the capacity of thymopoietin and other thymic factors to preserve or reconstitute the immune response; the effect of caloric restriction on immune function and life span; suppressor T cell activity; B lymphocyte function during aging;

and the pathogenesis of amyloid and autoimmunity.

Several research activities and findings are discussed below:

1) We know that at least one group of tissues, immunohemopoietic stem cells, are capable of functioning normally long beyond the lifespans of their donors. A lifetime of normal functioning has much less effect on stem cell lines than does a single transplantation. This work cautions that in elderly people, as in the mouse, losses of immune responses with age may not be intrinsic to stem cell lines.

2) Studies of the effect of 2-mercaptoethanol on a separated lymphocyte cell population indicate that the action of 2-mercaptoethanol may be to prevent an age-related increase in the suppressive effect of T-cells. Vitamin E, a natural antioxidant, when incorporated into the diet, delayed the age-related decline of immune function in mice.

3) Research on the evaluation of age and drug induced inflammation is underway. Studies in rats show that oral feeding of 6 sulfanilamidoinazole (6-SAI) causes an inflammatory arthritic lesion in the hind joints. Sulfonamide induces arthritis in 27, 50, and 100% of 3, 6, and 12 month old rats, respectively. The rats become highly sensitized to endotoxin and colchicine. Rats younger than 3 months are unaffected. This is an extremely important model showing the relationship of age, drug effects, and arthritis.

4) Dysfunctions of the immunoregulatory process (age-related increase in suppressor activity) are major factors in immunosenescence. One group of investigators has discovered a cell capable of suppressing the immune response which appears in the regenerating spleen of mice treated with the cytotoxic drug cyclophosphamide (Cy). This suppressor cell appears to be different from suppressor T cells, and is not classifiable as a T cell, B cell or macrophage. This special suppressor activity increases with advancing age in both long-lived mice and autoimmune-prone NZB mice. It is believed that the increased cell activity reflects an attempt by the animal to combat an ongoing autoimmune process.

5) Studies on the effects of caloric restriction on immune function, disease patterns, autoimmune manifestations, and lifespan are also underway. These studies should be an important clarifying step in removing dietary restriction studies from involvement with phenomena of early development and delayed growth. Furthermore, this research may have practical value because, at least in theory, such dietary restriction could be instituted in adult humans, with immune rejuvenation and extension of maximum lifespan.

6) Other findings strongly suggest the existence of a tricornered relationship between the main histocompatibility complex, DNA-repair capacity, and maximum lifespan potential. If so, then the immunological theory of aging and the DNA-repair theory, two of the leading current theories about why animals age, can be amalgamated. The application of known biochemical, genetic, and immunological information related to the main histocompatibility complex and DNA-repair mechanisms may also hopefully open a new area of research for a number of disciplines.

7) Cyclic nucleotide alterations in lymphocytes in Down's syndrome and in aging and normal humans are now apparent. A major defect in cyclic nucleotide function might be expected to have far reaching influences, not only on the lymphocyte system but possibly on other systems as well. This is perhaps the first step in what may be a fertile new area of aging research.

8) More than 200 subjects between the ages of 62 and 87 have had their immune status quantitated. The following alterations in immune parameters were found in the elderly population studied: (a) the third component of Complement was found to be significantly reduced; (b) the subsets of T cells were altered (T-gamma was increased, T-MU was decreased, and T-alpha was decreased); (c) circulating immune complexes were markedly increased; (d) PHA-induced proliferation of lymphocytes was markedly impaired; (e) autoantibodies were significantly increased.

9) Progeria is a rare genetic disease, with some clinical features of accelerated aging. Premature and accelerated vascular pathology is a manifestation of aging. Fibroblasts from 9 patients with this disorder show as a group a somewhat shorter in vitro lifespan than do fibroblasts from normal individuals. However, the variability among the nine strains studied was very great, with some strains reaching the expected 50 generations. Extensive studies of immune function in these patients have not revealed the age-associated defects in immunological function found in aged normal subjects. Progeria does not appear to be a model of normal aging in man.

Training within the Immunology Program addresses broad but fundamental questions pertaining to the role of the immune system in the manifestation of various diseases of aging. Trainees are currently working on studies on immune processes mediating the onset of autoimmune wasting diseases in animals and man which are thought to produce cardiovascular disease, vascular damage, kidney disease, rheumatoid arthritis, and lupus erythematosis. Others are engaged in research on basic immunological processes in aging subjects which may contribute to the increased appearance of neoplasia. The ultimate goal of the training program is to provide advanced and intensive training for qualified predoctoral and postdoctoral candidates in the immunobiology of aging.

Over 60 prominent immunologists from the United States, Italy and the Netherlands met at an international workshop on "Immunological Aspects of Aging" on May 7-8, 1979. Papers covered a wide range of topics relevant to the immune system, from phylogeny and ontogeny of the immune response to clinical studies. Several papers discussed the loss, with increasing age, of the ability to maintain normal immune function. There was discussion as to how restricted caloric intake and diets low in fat and high in protein and fiber may reduce the occurrence of autoimmune disease in man. A major paper discussed the relationship between aging and murine lupus, an autoimmune disease found in mice. Some papers surveyed research on diseases that offer special insights on the immunology of aging.

An announcement encouraging individual research initiatives, postdoctoral fellowships, and training programs in Immunology was published in the NIH Guide for Grants and Contracts, Vol. 8, No. 9, July 6, 1979. Research problems may address the following: (1) the macromolecular synthesis and regulation of lymphocyte activity; (2) the response of the immune system to biochemical stimuli; 3) the role of nutrition in modulating the antibody response; (4) the interaction between the immune system and the endocrine system; (5) the etiology and pathogenesis of amyloidosis and autoimmunity; (6) the immune response to infectious diseases; and (7) the utilization of existing and new animal models to study the induction and mitigation of age-related pathologies.

Pharmacology

In 1974 the per capita health cost for an older person was nearly three times that for a younger adult. A substantial proportion of that cost is due to expenditures for medication. It has been estimated that the elderly who comprise approximately 11% of the population use about 25% of the drugs produced. Relatively little is known about changes in drug effects in relation to the age of the individual and yet drugs are being administered more extensively to patients as they grow older. It is obvious that with increasing age there is a greater likelihood of illness, and therefore, a greater need to use therapeutic agents frequently on a chronic basis.

Drugs may behave differently in the aged than in the healthy young individual on whom most information has been acquired. Because of the many physiological, and perhaps pathological alterations occurring with age, it is essential that basic and clinical research be undertaken to evaluate the safety and efficacy of drugs being administered to the elderly. The possibility of undesirable pharmaceutical complications such as adverse drug reactions and drug-drug interactions may occur more frequently. Physiological impairment of the heart, blood vessels, kidneys, digestive tract, and nervous system among other organs are prevalent in the elderly individual. It is hoped that an NIA-supported program in Pharmacology will illuminate the aging processes as well as provide leads to more effective therapies.

Current studies are related to the effects of Vitamin C on pharmacokinetics in the elderly, the role of lipids in drug metabolism, physiologic responses of the aged to anesthesia, the effects of chronic utilization of therapeutic drugs; and the role of biological membranes in drug metabolism.

Research on drug bioavailability is being studied by measuring the bioavailability of certain test compounds (tetracycline, bretylium, tosylate, riboflavin, and sulfisoxazole acetyl) in middle age and senescent rats. Each compound explores a different aspect of GI absorptive capacity. The results indicate no marked differences between the middle aged and senescent groups in their ability to absorb orally administered drugs.

Other research is underway to make use of pharmacological approaches to elucidate changes occurring in membrane function with age in the heart, blood vessels, nervous system and kidney. The aim is not only to explore the relationship between changes in membrane function and the aging process but also to determine how pharmacological agents may modify, retard, or reverse the processes involved. If aging occurs as a result of changes in membrane receptor mechanisms, then such changes may result from alteration in the cellular environment and input to the cell. It now appears that aging involves a process which is related to the synthesis of membrane-bound receptors and that alterations in this process become evident with age because neurotransmitter and neuroendocrine input to the cell is modified.

Some research focuses on studies of the behavioral and biochemical effects of neuroleptic (anti-psychotic) drugs in young, mature and old rats. The overall aim is to investigate whether there are differential consequences of chronic neuroleptic administration in younger versus old animals, with the ultimate hope that this information will help provide evidence of the differential consequence of anti-psychotic treatment in geriatric patients. This research may also provide clues to explain some of the increased prevalence of tardive dyskinesia, a long-term side effect of neuroleptic drugs, in older psychiatric patients, and point to ways to remedy this situation. This investigation advances research in several important ways: (1) It further delineates important biochemical changes in the aging rat brain which may account for deficits in function in older age humans and differences in responses to psychotropic drugs in geriatric patients. (2) It extends work on brain receptor binding, which has become an important technique in psychopharmacology, and shows important decreases in adrenergic and dopaminergic receptors in the aging brain. (3) It provides leads which may help explain the increased prevalence of tardive dyskinesia in geriatric psychiatric patients. (4) It shows that some receptor changes in response to long term administration of neuroleptic drugs which are found in older animal brains are not found to the same extent in younger animals.

The major objective of the training program is to develop manpower with an appreciation for the problem of pharmacokinetic changes associated with the aging process, the possible clinical and therapeutic implication of these changes, and approaches to assessing specific basic and clinical problems.

Currently an announcement encouraging research in pharmacology and aging is in circulation. Research and training applications may be directed towards the discovery and elucidation of the influence of physiological and pathological changes on drug safety and efficacy.

Intermediary Metabolism

Research currently being supported can be divided into the following categories: (1) age-associated changes in the structure and function of connective tissue components such as collagen and elastin; (2) age-associated changes in the biosynthesis of proteins and other macromolecules; and (3) the effect of age on mitochondrial structure and function. The following are highlights of research progress achieved during the past year.

It has recently been shown that in metabolically stable proteins such as the proteins of tooth enamel, dentine, and collagen, the L-aspartyl residues undergo a slow racemization with time, producing significant quantities of D-aspartic acid. Since this reaction occurs throughout the lifespan of an animal, it is possible to calculate the "biochronological" age based on the amount of D-aspartic acid which has accumulated. This technique has been tested by demonstrating, with various mammals, that the ages derived from racemization are in close agreement with those obtained from birth records or other age-related information. In addition to being of value for the determination of age, this research has implications for age-associated changes in structure and function of tissue containing metabolically stable proteins.

Another important area being examined is concerned with the effect of aging on the hierarchical organization of collagen superstructure and the relationship of these age-associated changes to changes in the mechanical behavior of tendon. It has been observed that aging changes the entire spectrum of the mechanical function of tendon, particularly in response to extreme conditions when damage and fracture have occurred. The results of these studies indicated that although there was an age-associated effect of physical stress on collagen fiber superstructure, the characteristics of individual collagen fibrils as determined by electron microscopic observation was not affected. In another aspect of the same study, age-associated changes in the susceptibility of collagen to digestion by collagenase has been examined. The results show a progressive, age-associated resistance to digestion using collagen isolated from heart, tendon, and dura mater. This study is therefore attempting to correlate changes in the physical and mechanical properties of collagen and other connective tissue with changes in their chemical and metabolic states as a function of age.

One explanation that has been offered for the progressive worsening of physiological function of aged organisms is the accumulation of ineffective or damaged organelles or macromolecules, particularly in postmitotic cells. An investigation of this phenomenon in aging dogs and mice however, has failed to demonstrate the presence of detectable levels of either defective adolase or defective superoxide dismutase. Furthermore, studies on protein catabolism in perfused mouse and rat liver have revealed no differences in the rate of degradation of either normal or abnormal proteins. These data suggest that if the biosynthetic mechanism in old cells produce defective proteins, as has been speculated, such defective proteins can apparently still be degraded by the normal catabolic mechanism whose efficacies are not noticeably diminished.

A closely related study is examining age-associated changes in the frequency with which amino acid substitutions occur during protein synthesis. An increase in frequency observed with aging could be one mechanism which would lead to increased formation of faulty protein molecules in older cells. Although this work is not as yet complete, the interesting substitution, valine for threonine, has been observed. This work will hopefully lead to more definitive answers about age-associated changes in the frequency of amino acid substitutions which occur during protein synthesis.

Diabetes

Diabetes millitus is a major health problem afflicting the elderly. It is highly prevalent in persons age fifty and above and may range in severity from a mild glucose intolerance, which can be regulated through proper dietary control, to an extreme insulin-dependent disease.

Although the high incidence of diabetes in the elderly has been observed for several years, its underlying cause is still unknown. For instance, it is not clear whether the loss of glucose tolerance with age is true diabetes, or simply a nonpathological manifestation of normal aging. These uncertainties in part reflect the lack of basic knowledge about the biochemical and physiological changes which are the underlying cause of diabetes. This is an especially important problem in the elderly where the changes leading to the development of diabetes are superimposed upon the physiological processes which accompany normal aging. In order to identify the causes of diabetes in the elderly, it is essential to understand more fully the basic processes and mechanisms underlying the disease and its metabolic and endocrinologic complications.

The Diabetes Program supports research whose major focus is investigating the underlying causes and mechanisms for the development of diabetes and its complications in the elderly, as well as research on developing better and improved methods of treatment. Additional areas of interest include epidemiological studies of diabetes and glucose intolerance in the elderly as well as studies that examine the psychosocial factors which affect elderly diabetics. The following highlights are results which have been obtained from research grants supported by the Diabetes Program over the past year.

Although insulin has assumed a central role in carbohydrate metabolism, age-associated changes in the levels and/or action of other hormones which also affect glucose utilization may play a role in the development of diabetes in the elderly. A particular example currently being investigated is a family of polypeptide hormones named somatomedins, whose action is important for the growth of bone, muscle, and other tissues. It recently has been demonstrated that there is an age-related decrease in the levels of circulating somatomedins in old animals. At the same time, however, there is no decrease in the circulating levels of growth hormone, which is primarily involved in stimulating somatomedin secretion. These results suggest that age-related changes in muscle mass may result from lowered levels of the principle muscle growth stimulating hormone, rather than from a change in the muscle itself.

Another area of current interest is concerned with an examination of the mechanisms by which certain insulin-dependent processes undergo a progressive change during aging. In this regard it has been found that utilization of glucose is impaired upon aging in male Sprague-Dawley rats. In addition, glucose stimulated secretion of insulin is impaired during aging both *in vivo* and in isolated, perfused islets of Langerhans from rats. These results indicate that aging Sprague-Dawley rats may therefore provide a useful animal model for studying maturity onset diabetes in the human.

NIA Annual Report
October 1, 1978 through April 15, 1979

Molecular and Biochemical Aging Program
Biomedical Research and Clinical Medicine

Contract Number: N01 AG 06-2122

Contract Title: A Study of Lymphocyte Function and Serum Immunoglobulin Concentration During the Lifespan of Individual Mice

Contractor: Cornell University Medical Center, New York, New York
(Contract Officer: Dr. Marc E. Weksler)

Money Allocated: $22,425

Objectives: The purpose of the contract was to assess immune reactivity by measuring the proliferative response of blood lymphocytes in culture and by measuring the plasma concentration of immunoglobulins. The aims of this study have been met.

Significance to aging research: Although defects in immune function of aged mice have been repeatedly demonstrated, the survival value of immune reactivity has never been shown to be either positive, negative or to have no effect. That is, do animals with vigorous immune reactivity die early while those possessing lower reactivity survive longer? Or does immune reactivity decline with age and those with the most impaired immune function die first? Either hypothesis would result in the observation of impaired immune function observed in all aged mice.

Results: A longitudinal, lifespan study of immune function in three strains of mice was performed. Lymphocyte proliferation declined while serum immunoglobulin concentration increased as individual animals aged. These data confirm conclusions drawn from cross-sectional studies of immune function in mice. This was the first lifespan study of immune function in experimental animals. These data also provide immune functional parameters for the NIA mouse colony at the Charles River Laboratories.

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Molecular and Biochemical Aging Program
Biomedical Research and Clinical Medicine

Contract Number:	N01 AG 09-2100
Contract Title:	Longitudinal Study of Immune Function in Thymic Hormone Treated Mice
Contractor:	University of Alabama, Birmingham (Contract Officer: Dr. Raymond N. Hiramoto)
Money Allocated:	$158,154

Objectives: The objective of this contract is to evaluate the effect of thymic hormone preparations on the derangements in immune function observed during aging in mice. The impaired proliferation of T-lymphocytes in culture suggests a loss of thymic dependent immune function. The increased prevalence of autoantibodies in aged mice also points to an altered balance between helper and suppressor T-lymphocytes. Whether these derangements in immune function can be delayed or prevented by thymic hormone supplementation and whether lifespan thereby can be extended is not known. This contract will support a longitudinal lifespan study of immune function in untreated mice and in mice treated with thymic hormone preparations. The effects are being examined with four (4) thymic hormones in C57BL/6, A/HeJ and CFW strains of mice. These studies will be carried out in collaboration with Drs. Gideon Goldstein (Director of Immunoscience, OrthoPharmaceutical Corporation, NJ), the discoverer of thymopoietin; Allan Goldstein (Department of Biochemistry, George Washington University Medical School, Washington, D.C.), the discoverer of thymosin; Jean Francois Bach (Hospital Necker, Paris, France), the discoverer of facteur thymic serique; and Nathan Trainin (Department of Cell Biology, The Weizmann Institute, Rehovot, Israel), the discoverer of thymic humoral factor.

Significance to aging research: Because the increase in the incidence of immunologic dysfunction, autoimmunity and cancer is intimately related to aging, this contract will evaluate the pathological changes in animals on long term hormone treatment. Thymic hormone replacement may alter the pathologic state associated with aging in man.

Results: Contract just under way.

BIOPHYSIOLOGY AND PATHOBIOLOGY AGING PROGRAM

The Biophysiology and Pathobiology of Aging Program (BPAP) is structured to foster research on aging in the following disciplinary areas: neuroscience, geriatric medicine, nutrition, endocrinology, exercise physiology, and animal models. These program elements have a complementary scientific interrelationship therefore; they are integrated within a single program in the Biomedical Research and Clinical Medicine Program (BRCM) of the National Institute on Aging (NIA). The primary thrust of the program is to encourage the development of integrated research in clinical medicine and basic biomedical sciences. The animal models subprogram constitutes an important part of the program which is a focal resource not only for the BPAP but for the whole Institute.

Recent Applications and Funding Data:

Table I
Reviewed, Approved, and Funded NIA Competing Applications for FY 79* by Funding Mechanism

Biophysiology and Pathobiology Aging Program

Overall View

Funding Mechanism	Total Applications		Applications Approved by IRG		Funded by NIA	
	Number	Amount	Number	Percent	Number	Percent
Research Grants						
R01's	182	11,621,041	115	63%	40	22%
P01's	6	1,591,870	5	83%	4	67%
Research Training Grants						
T32's	2	185,080	2	100%	1	50%
Fellowships						
F32's	12	174,000	9	75%	4	33%
Research Career Development Awards						
K04's	5	161,961	2	40%	1	20%
Other (R21, R23)	77	3,499,049	34	44%	17	22%
Total	284	17,233,001	167	59%	67	24%

*This table includes those grants which have already been funded and those which have been approved and will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for August 10 mailing to the Council Working Group.

TABLE 2

Approved NIA Competing Research Project Applications for FY 79* by Normalized Score

Biophysiology and Pathobiology Aging Program

Overall View

Normalized Score	Number of Applications	Percent of Total
100-125	0	0
126-150	2	1.4%
151-175	3	2.0%
176-200	9	6.2%
201-225	6	4.1%
226-250	20	13.7%
251-275	23	15.7%
276-300	26	17.8%
301-350	29	19.9%
351-500	28	19.2%
Total	146	100%

* This table includes those grants which have already been funded and those which will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for the August 10 mailing to the Council Working Group.

NEUROSCIENCE OF AGING SUBPROGRAM OVERVIEW:

The Neuroscience of Aging Subprogram supports research which focuses on the relationship between aging and the nervous system. The program fosters a spectrum of research within neuroscience (e.g., neurobiology, neuroanatomy, neurochemistry, neuropharmacology, neuroendocrinology, neuroimmunology, neurophysiology, neuropsychology, histochemistry, histology, electrophysiology, and, biophysics) whenever the focal point of investigation is the aging process and concomitant changes in the structure and function of the nervous system.

The specific aim of the program is to encourage the development and dissemination of information in both basic and clinical neuroscience. To attain this objective, the program supports the formulation of new theories and development of novel perspectives, methods, and instrumentation for the study of the aging nervous system. The long-range goal of the program is to facilitate the translation of basic scientific information into practical applications for the treatment and care of the elderly with neurological infirmities.

Selected Research Progress Summaries:

Dr. Landfield's most recent results indicate that some aspects of the brain aging process may be reduced in the adrenalectomized rats.

It appears that in those aging Fischer rats in which the adrenal glands were surgically removed at 14 months of age, and which were subsequently maintained for 5 1/2 months, there are fewer morphological signs of brain aging at 20 months of age than are found in control animals of the same age. The control animals (maintained for the same period) included one group of intact animals, and another group of adrenalectomized animals that were given a high-replacement dose of the adrenal (stress) hormones.

If further analyses confirm these initial indications, this study will represent the first evidence of an experimental retardation of the development of morphological correlates of brain aging. However, it needs to be emphasized again that the data are still very preliminary, and considerable additional analyses will be required before any firm conclusions can be reached.

These data seem particularly exciting, because to this point they are highly consistent with the hypothesis that hormonal systems (e.g., the adrenal cortex system) modulate the rate of some aspects of brain aging (see Landfield, in Parkinson's Disease II: Aging and Neuroendocrine Relationships, Finch et al., (eds.), Plenum, 1978). Additionally, these preliminary findings on effects of adrenalectomy on brain aging are consistent, with prior studies. That is, they are consistent with an earlier Landfield study indicating that levels of adrenal system activity are positively correlated with the degree of one brain aging measure in spontaneously aging individual rats (Landfield et al., 1978, Science) as well as with the study indicating that long-term administration of adrenal hormones can accelerate the development of at least one measure of brain aging (Landfield, et al., 1978, Soc. Neurosci. Abstracts).

If further research does in fact bear out a role of adrenal hormones in brain aging, then we may have an opportunity to make an important impact on the health of aging individuals in the not too distant future. That is, it seems quite possible that present levels of adrenal (stress) hormones in humans are higher than is necessary for modern man. If we could pharmacologically reduce these levels, we might be able to reduce the rate of brain aging (assuming the initial results are confirmed). At present, there is of course considerable interest in the possibility that the brain control systems which regulate endocrine activity may deteriorate with age (see Finch in Parkinson's Disease II: Aging and Neuroendocrine Relationships, Plenum, 1978). Such altered hormonal regulation can lead to widespread physiological imbalances and perhaps to many of the most deleterious aspects of the senescent phase (e.g., senile brain decline, muscular wasting, osteoporosis, atherosclerosis, diabetes, etc.). It seem feasible, therefore, that reducing the rate of brain aging could make the sensecent phase one of considerably greater mental vigor, muscular strength and general health than is presently the case. Of course, considerable work on the possible deleterious side effects of hormonal manipulation would be necessary prior to any application to human health problems.

The results from another major project on brain aging in which Dr. Landfield is involved (i.e., brain neurophysiology during aging) also may be of some interest. He has found that a highly specific neurophysiological age change, i.e., depression of nerve cell firing rate is highly correlated with the ability of aged animals to show evidence of retention of an avoidance task which they had previously learned (r=+.76). That is, the more prominent was the memory retention deficit, the more severe was the neurophysiological deficit. These results suggest that the neurophysiological changes observed may be directly relevant to behavioral decline with age, and that if we can find pharmacological agents which specifically counteract this neurophysiological deficit than such agents might also counteract, at least temporarily, senile behavioral change.

In a recently completed study, Dr. Marshall has found that a deterioration of neurotransmission at brain dopa-minergic synapses appears to contribute to some of the movement disorders of advanced age. In his studies he has demonstrated that two-year old rats show profound deficiencies in their ability to sustain vigorous and effective swimming during a 15-minute test. However, these rats swim as well as young adult animals when they are given compound (apomorphine or L-dopa) that increase brain dopamine receptor stimulation.

Aged rats given apomorphine show a dramatic rejuvenation of their swim performance. Even the lowest dose (0.25 mg/kg) induces senescent rats to swim more vigorously and successfully than young adults. A moment-by-moment analysis of the swimming reveals that this improvement is attributable to a prolongation of vigorous and effective swimming. Like young adults, the apomorphine-treated aged animals adopt a position nearly horizontal to the water surface. They plane across it, keeping their heads above water for most of the 15-minute test. Apomorphine also significantly enhances both measures of swimming in young adult rats. However, this improvement is less marked in the younger animals, and occurs at doses higher than those required to improve the swimming of aged rats, as indicated by the significant interaction between age group and dose for both measures.

In order to determine whether the restored swimming behavior of aged rats would occur following administration of another compound that increases brain dopamine receptor stimulation, the experiment was repeated using the biosynthetic precursor of catecholamines, L-dopa. When administered systemically, this amino acid is converted to dopamine in the brain by the enzyme aromatic amino acid decarboxylase.

The results showed that L-dopa rejuvenates the swimming of aged rats even at the lower dose. The aged animals swam with improved vigor and success, comparable to that seen in young adults. Only at the higher dose is the swimming of the younger rats improved, and even then only in the success measure. The interactions between age groups and dose were highly significant for both measures.

These experiments demonstrate for the first time that aged rats suffer from severe disturbances of movement. Their swimming performance deteriorates rapidly as the test continues, suggesting that their movement dysfunctions are particularly apparent when prolonged, strenuous movements are required. Not only do the sensescent animals swim with impaired vigor and success, they appear unable to develop an effective strategy for coping with the stress of a prolonged swim. Each of these impairments is reversed by dopamine agonist compounds.

The dramatic rejuvenation of swim performance by compounds that enhance brain dopamine receptor activity holds great potential significance for understanding the movement distrubances of aging. First, their efficacy in restoring swimming indicates that the poor swim performance of aged rats in the drug-free condition is not attributable solely to peripheral factors, such as diminished muscle mass or increased adiposity; instead, the present findings suggest a CNS origin of this deficit. In particular, the poor swim performance of the aged animal appears linked to age-related changes in neurotransmission at brain dopaminergic synapses. Numerous investigations have indicated abnormalities in central dopamine synapses of advanced age humans and animals. Given this background of subnormal dopamine synaptic activity in sequences, low doses of apomorphine or L-dopa appear to facilitate movement by normalizing central dopamine receptor stimulation.

Dr. Cote who has been studying the activities of enzymes involved in the synthesis and degradation of catecholamines reports observing a sharp decrease in tyrosine hydroxylase and Dopa decarboxylase activities in the extrapyramidal nuclei occurring in middle-age subjects (around 40 years of age). On the other hand, both the A and B forms of MAO increase in activities with age.

Glutamic acid decarboxylase activity drops with age but not to the degree seen with tyrosine hydroxylase and Dopa decarboxylase. Acetylcholinesterase and butyrylcholinesterase activities decrease slightly with age in human brain.

Dr. Cote also reports that one of the characteristic changes associated with aging, in some model systems, is the presence of increasing amounts of "altered" enzyme molecules with a resultant reduction in catalytic efficiency.

In Dr. Robbins' laboratory, the major effort up to the present has been the development and evaluation of techniques, most of which are now ready for application. Methods have been developed to record and analyze by computer spontaneous and evoked nerve activity at neuromuscular junctions of young and aging mice. It is expected that within a short time the basic outlines of correlative synaptic physiology and morphology as a function of age should be well advanced. Other studies in the laboatory on reactivity of muscle to loss of innervation in terms of cellular proliferation and early enzymatic alterations will be pursued in order to elucidate the role of the nerve in long-term regulation of muscle. Two major findings were that enzyme responses to denervation ensue rapidly after nerve terminal failure and are not simply dependent on disuse. Also, a unique set of extrinsic cells were found to be the major source of cell division after denervation. Alterations of these neurotrophic regulators of muscle properties may help to explain alterations of nerve and muscle properties in aging.

In Dr. Lasek's laboratory, the purification of neurofilament proteins in mammals has been successful, and the phosphorylation reactions of these purified proteins have been characterized biochemically. Antibodies to the subunits have been obtained, and will be used to further analyze the phosphorylation reaction and to further purify neurofilaments by affinity chromatography. In addition, antibody studies showed that neurofilaments in Myxicola, a polychete, differ from the filaments found in glia cells. These studies have opened the way to an analysis of the functional role of neurofilaments, and may be of importance in understanding the filamentous neuronal tangles characteristic of Alzheimer's senile degeneration. In other studies on extruded anxoplasm of the squid, it was shown that neurofilaments remaining after most cytoplasmic proteins were allowed to diffuse away, were sufficient to maintain the cytostructure of axons. Further work on the axoplasmic skeleton will supply a biochemical basis for analyzing morphology of axons and their abberations. Finally, growing out of this project, a collaborative effort of Drs. Lasek and Gulley led to the discovery of an extremely rapidly transported and turned-over protein in the auditory system.

Dr. Gulley, in collaboration with Dr. Lasek, found that two properties which were prominent components of the fast component of axonal transport, were transported rapidly to the cochlear nucleus. One of these disappeared within five hours. The biochemical and physiological significance of this protein, and the effects of use and disuse, will be explored next. In other studies, Drs. Gulley and Bird have shown that local kainic acid led to selective destruction of cells in the cochlear nucleus. This finding supplies indirect evidence that the postsynaptic cells have glutamate receptors. Furthermore, it's been shown that kainic acid effects do not depend on the presence of the presynaptic terminal. Further, freeze-fracture characterization of this effect may reveal plasticity of the receptor proteins in the auditory system.

Dr. Singer's group have discovered that ependymal processes guide showing axons during embryogenesis and in regeneration of adult newt central nervous systems. The detailed ultrastructure of these pathways will be examined next. This work may have an important bearing on growth and regrowth of the normal and aging axons in the central nervous system. In another area, Dr. Singer's group begun to study regeneration of fingers in the immature monkey and the neurotrophic role of the nerve in this process. Such neurotrophic interactions may underly the poor regeneration of body parts characteristic of different ages or species.

The studies of Dr. Selkoe seek to elucidate the role of major class of neuronal proteins, the fibrous protein both in normal aging and in the pathogenesis of the late-in-life dementias. Dr. Perl has been attempting, through the use of a scanning electron microscope with an electron-probe analysis, to determine the elemental contents of tangle-bearing neurons in the brain. His preliminary results indicate that tangle-bearing neurons contain focal intranuclear concentrations of aluminum and silicon. Dr. Davis, on the other hand, is more interested in understanding the reasons for the losses of enzymes of control cholinergic neurons in human aging and in Alzheimer's patients.

Dr. Sylvia has improved upon a noninvasive optical monitoring technique to continuously measure changes in brain activity, oxidative metabolism and circulatory parameters. This study will enable to correlate the process of aging brain homeostatic malfunctions mediated by changes in brain oxidative metabolism and changes in microcirculation. Dr. Katzman's studies seek to determine the specific genetic environmental and metabolic risk factors in Alzheimer's Disease. This study entails a cross-cultural case control study which utilizes epidemiological and biological approaches.

In addition to these investigators' initiated projects, the extramural program at NIA supports four major training programs which provide specialized training for both basic and clinical investigation as Alzheimer's Disease.

The results of Dr. Fox's studies have shown that both cerebral atrophy (by computerized tomography) and chronological age had significant, independent effects upon verbal learning, but only cerebral atrophy significantly affected span of immediate recall (digit-span). This suggests: (1) that adult age-group differences in verbal learning cannot be accounted for entirely on the basis of differences in degree of cerebral atrophy, (2) that chronological age and cerebral atrophy have additive rather than interactive effects upon certain aspects of memory (i.e., cerebral atrophy has a similar effect on memory in 50- to 69- and 70- to 89-year old groups, although older patients without atrophy perform more poorly than younger patients without atrophy and older patients with atrophy perform more poorly than younger patients with atrophy), and (3) different aspects of memory functioning (span of immediate recall versus verbal learning) are differentially affected by cerebral atrophy.

In another study designed to determine the nature and relative strength of various predictors of different aspects of cognitive functioning in a large sample of patients suspected of presenile or senile dementia, Dr. Fox reports finding that: (1) EEG slowing is the strongest and most

Other Major Developments:

1. On June 1 and 2, 1978, the NIA sponsored a Workshop on Sleep and Aging in Bethesda, Maryland. This meeting brought together an internationally known group of investigators with an active interest in in sleep research, problems of the elderly, and the physiology, psychology, and pathology of aging. They identified and discussed the important gaps in our knowledge concerning the sleep problems of the aged, and defined for the scientific community the most promising areas of investigation. The summary of the Workshop's proceedings was published in Sleep, Vol. 1, No. 3, 1979, by Raven Press. A program announcement based on the proceedings of this workshop is being planned.

2. On August 9 and 10, 1979, a seminar on the "Hearing Problems of the Elderly" will be held jointly by NIA and NINCDS. The topics to be discussed include: (1) epidemiological issues of age-specific norms for hearing loss; (2) economic aspects of hearing loss in the aged; (3) diagnostic problems and issues; (4) treatment and management of patients with hearing problems; (5) prosthetic devices and sensory aids. The conference is intended to identify gaps in our knowledge regarding Presbycusis. It is expected that a program announcement will result from this seminar.

3. An interagency agreement between NIA and NASA was signed. The purpose of this effort was to facilitate the transfer of NASA-developed technology to biomedical application, in particular, to meet the special needs of the elderly and the handicapped.

4. Preliminary discussions are taking place with NINCDS to establish a colony of old monkeys for studies related to the Neuroscience Subprogram.

Recent Applications and Funding Data:

Table I
Reviewed, Approved, and Funded NIA Competing Applications for FY 79* by Funding Mechanism

Biophysiology and Pathobiology Aging Program

Neuroscience

Funding Mechanism	Total Applications		Applications Approved by IRG		Funded by NIA	
	Number	Amount	Number	Percent	Number	Percent
Research Grants						
R01's	56	3,256,775	38	68%	14	25%
P01's	3	667,022	3	100%	2	67%
Research Training Grants						
T32's	-					
Fellowships						
F32's	7	101,500	5	71%	4	57%
Research Career Development Awards						
K04's	2	60,489	1	50%	1	50%
Other (R21, R23)	17	657,647	12	71%	4	24%
Total	85	4,763,433	59	69%	25	29%

*This table includes those grants which have already been funded and those which have been approved and will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for August 10 mailing to the Council Working Group.

TABLE 2

Approved NIA Competing Research Project Applications for FY 79* by Normalized Score

Biophysiology and Pathobiology Aging Program

Neuroscience

Normalized Score	Number of Applications	Percent of Total
100-125	-	
126-150	1	2%
151-175	1	2%
176-200	2	4%
201-225	3	6%
226-250	8	17%
251-275	5	10%
276-300	6	13%
301-350	9	19%
351-500	13	27%
Total	48	100%

* This table includes those grants which have already been funded and those which will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for the August 10 mailing to the Council Working Group.

GERIATRIC MEDICINE SUBPROGRAM OVERVIEW:

The Geriatric Medicine Subprogram is aimed at the development and support of clinical research on the medical problems of the elderly that may influence maintenance of health, quality of medical care, establishment of health care requirements, medical practice, diagnostic, surgical, and preventive procedures.

The gradually shifting distribution of the population in favor of the older age groups require the development of research programs on medical problems unique to the aged. Studies are needed to define the current basis for medical care provided to the elderly. Also, there is a need for a systems analysis of medical care provided to the elderly that will prevent or moderate, rather than exacerbate, long-standing chronic impairments associated with aging. Basic to these studies is the need for epidemiologic analysis to identify medical, physical, and psychological risk factors in the aged and the relative risks to benefits of therapeutic interventions in the aged patient.

Other major developments:

Oral Motor Behaviors: NIA-NIDR Conference on Dental Problems

1. The NIA was a cosponsor of this NIDR-planned conference which was held on May 9 and 10, 1979. This conference, in part, dealt with recommendations for research on dental problems in the elderly. A book is being planned for publication in the fall of 1979.

2. In September 1978, the National Institute on Aging announced the availability of a Geriatric Medicine Academic Award which had the dual purpose of improving the quality of curricula in geriatrics and of fostering research and careers in the field of aging.

The Institute initiated the Geriatric Medicine Academic Award Program to provide a stimulus for development of a curriculum in geriatric medicine in those medical schools that do not have one and to strengthen and improve the curriculum in those schools that do. Awards provide support to individual faculty members for their educational development, and for implementation of the curriculum in geriatric medicine.

Recent Applications and Funding Data:

Table I
Reviewed, Approved, and Funded NIA Competing Applications for FY 79* by Funding Mechanism

Biophysiology and Pathobiology Aging Program

Research Geriatric Medicine

Funding Mechanism	Total Applications		Applications Approved by IRG		Funded by NIA	
	Number	Amount	Number	Percent	Number	Percent
Research Grants						
R01's	12	968,874	6	50%	3	25%
P01's	-					
Research Training Grants T32's	-					
Fellowships F32's	-					
Research Career Development Awards K04's	1	35,975	-			
Other (R21, R23)	30	1,581,558	11	37%	5	17%
Total	43	2,586,407	17	40%	8	19%

*This table includes those grants which have already been funded and those which have been approved and will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for August 10 mailing to the Council Working Group.

TABLE 2

Approved NIA Competing Research Project Applications for FY 79* by Normalized Score

Biophysiology and Pathobiology Aging Program

Research Geriatric Medicine

Normalized Score	Number of Applications	Percent of Total
100-125	-	
126-150	-	
151-175	1	10%
176-200	2	20%
201-225	-	
226-250	-	
251-275	-	
276-300	4	40%
301-350	2	20%
351-500	1	10%
Total	10	100%

* This table includes those grants which have already been funded and those which will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for the August 10 mailing to the Council Working Group.

NUTRITION SUBPROGRAM OVERVIEW:

Our knowledge on clinical nutrition in relation to aging processes, disease prevention, and health maintenance is in a relatively primitive state. This is evidenced by the lack of adequate information on standards for the nutritional requirements of the elderly. The utilization of nutrients are largely unknown and those that are employed are extrapolations based on younger age groups. The primary and secondary effects of dietary deficiencies on decrements in physiological function are not known.

Except for lipid metabolism and the effect of a few vitamins and trace minerals, virtually nothing is known about the influence of nutrition on disease prevention or vigor in the aging adult. Deficiencies and excesses in some micronutrients such as vitamins, minerals, and drugs are known to be associated with central nervous system disturbances, to influence normal wound healing, affect metabolic processes and physiologic function. But the data necessary to establish functional relationships are missing. Many, if not most, of the debilitating conditions seen in the aging adult are related to a nutritional deficiency or excess. Until this information can be acquired and validated through basic and clinical nutrition research and integrated into preventive medical practice, the health of the aging adult will continue to be compromised.

Selected Research Progress Summaries:

Dr. Porta's studies use biochemical and morphometric analysis of: albuminglobulin ratios, levels of vitamin E, triglycerides, phospholipids and cholesterol, levels of triglycerides, phospholipids, cholesterol, DNA, RNA, protein, vitamin E, glutathione peroxidase in various organs such as, brain, heart and liver of animals sacrificed at various age groups. His results show that at each of the periods studied no significant differences between groups were generally detected in food intake, body weight gain, weights of liver, heart and brain, serum albumin-globulin ratios, organ concentrations of DNA, RNA, protein, triglycerides, cholesterol or phospholipids. On the other hand, it was unexpectedly found that after three months of dietary treatment all rats from the different groups became manifestly obese and that the levels of hepatic triglycerides were abnormally increased (about 4-fold over normal values). Since the magnitude of increase in hepatic triglyceride and the periportal lobular distribution of the histologically visible fat observed in these rats were similar to those found in rats with experimentally induced obesity, the most likely reason for the hepatic fatty changes is obesity.

While at each of the periods studied no significant differences between groups were found in the concentrations of cerebral, cardiac or hepatic protein, the levels decreased with age almost equally in all groups. These results appear to be at variance with those previously found by other investigators, at least in the case of cerebral protein.

A modest, but statistically significant hypotriglyceridemic effect of unsaturated fat diets was only observed in rats killed at 6 and 24 months. Unsaturated fat diets had also a significant hypophospholipidemic effect but only in rats killed at 6 months. Plasma cholesterol concentrations were unaffected by the type of dietary fat, but high levels of vitamin E appeared to have a transient hypocholesterolemic effect in rats fed diets containing a combination of saturated and unsaturated fat and killed at 12 months.

The concentrations of vitamin E in plasma as well as in liver, heart and brain generally maintained a direct relationship with the dietary levels of this vitamin, although were also variably modified by the type of dietary fat. On comparative basis (irrespective of dietary treatment) the magnitude of vitamin E concentrations had the following decreasing pattern: liver heart brain plasma. It was also found that in each of these tissues the concentrations of vitamin E increased with age. These findings appear to be in line with those previously reported by other investigators indicating that the tissue requirements of vitamin E decrease with aging.

The significance of these studies are that there are certain differences between groups which, hopefully will serve in the identification of the dietary regimen more beneficial for the retardatrion of aging changes and for the prolongation of the life span of rats.

The significance of these studies in relation to the general problem of aging and dietary factors is that they provide fundamental data on the biochemical and morphologic aspects of aging as well as indicate the influence of diet on life span.

Dr. Kritchevsky, who has been studying the relationship between lipid metabolism and aging, finds evidence that there are both age and strain differences in body and liver weight and in serum levels of lecithin-cholesterol acyl transferase (LCAT). Most of the other significant differences were age related. Aortic cholesterol levels were similar.

This systematic study of lipid metabolism in aging has shown that one strain of rats, Fisher 344, does not continue to gain weight after reaching 6-9 months of age. The lipid response in aging is somewhat slower in this strain of rat (compared to Wistar or Sprague-Dawley strains). Sphingomyelin in Wistar rat plasma increases with age.

Dr. Wagner has designed a study to obtain a better definition of normal values related to zinc nutriture in aging women. The evaluation of zinc nutriture is of particular importance in the aging adult because of the fundamental roles of zinc in tissue repair, protein synthesis, wound healing and appetite regulation.

An evaluation of the nutritional status of elderly individuals in a poverty area of Miami is now in progress. Extensive socioeconomic, dietary, clinical and laboratory data were collected from 193 individuals in this survey. Samples of hair and blood serum have been stored for zinc determination as soon as a new atomic absorption spectrophotometer is operational. Prior to the Miami project, a smaller scale survey of 53 participants in the Alachua County (Gainesville) Nutrition Program for the Elderly was conducted in order to determine nutrient intakes and hair zinc values.

Because of the recently reported role of zinc in the intestinal absorption of folacin polyglutamates (Tamura, T., M.T. Baer and E.L.R. Stokstad, Reduced absorption of folate polyglutamate in zinc-depleted man. Fed. Proc. 37:493, 1978) folacin status will be determined in the present study.

The findings from the survey of elderly Maimi individuals demonstrated widespread folacin deficiency [erythrocyte folacin was deficient (less than 140 mg/ml) in 68% of the subjects and low (140-160 mg/ml) in 11%]. Others have recently reported impaired absorption of folacin polyglutamates in the elderly (Baker, H., S.P. Jaslow and O. Frank. Severe impairment of dietary folate utilization in the elderly. J. Am. Geriatric Soc. 26:218-221, 1978). Therefore, the addition of folacin measurements to the present study should help to clarify the influence of zinc nutriture on the folacin status of aging women.

Dr. Zamenhof reports that chronic malnutrition considerably reduces life span. He has found that following chronic food restrictions over generations, adult female rats have a 40% reductions in half life as compared to the ad lib control. Following that time (age 9 months), a further rapid decline in survivors is found and at 26 months of age only 12% are still alive; in contrast, in the control group 38% are alive at 26 months of age.

The effects of prenatal (only) protein-calorie restriction on aging and survival are under investigation in this laboratory. Preliminary results indicate possibly a different pattern of loss than the one demonstrated in chronically malnourished animals. This group of rats had a minimal loss following 30 days of age (i.e., a reduction of juvenile incident of death).

In the same vein Dr. Forbes also has been investigating the effects of dietary restrictions on somatic growth, life span, rate of lipofuscin accumulation in the brain, and aging-related changes in acoustic startle response habituation and various quantitative characteristics of the electroencephalogram.

He has found that pre-weaning restriction resulted in a retardation of somatic growth which has persisted to nearly 2 years of age.

The longevity data reported by Dr. Forbes confirms numerous prior reports that dietary restriction may result in increased life span in laboratory rats. Whether this phenomenon is a result of a restriction-induced decrease in the rate of aging or is merely a reflection of decreased incidence of aging-related pathologies is not known. The studies Dr. Forbes proposes to conduct will provide information regarding the relationship between restriction-induced increases in longevity and the rate of aging-related changes in the brain. If it is found that aging and the onset of senescence is retarded by dietary restriction, a significant insight into the control of the aging process will have been achieved. If, on the other hand, it is found that increased longevity is not associated with decreased aging, these studies will have important technical implications for future studies of aging in laboratory animals.

Other Major Developments:

The NIA and NICHD jointly sponsored a conference on "Nutrition, Behavior, and the Life Cycle" which was held June 18-20, 1979 at the NIH Campus. The purpose of this meeting was to identify the interrelationships between nutrition and behavior throughout the life cycle. A summary of the proceedings of this conference is being prepared for publication, in addition both institutes are planning to issue a Program Announcement based on the recommendations of the participants.

Recent Applications and Funding Data:

Table I
Reviewed, Approved, and Funded NIA Competing Applications for FY 79* by Funding Mechanism

Biophysiology and Pathobiology Aging Program

Nutrition amd Metabolism

Funding Mechanism	Total Applications		Applications Approved by IRG		Funded by NIA	
	Number	Amount	Number	Percent	Number	Percent
Research Grants						
R01's	51	3,534,249	25	49%	8	16%
P01's	1	208,812	1	100%	1	100%
Research Training Grants						
T32's	-					
Fellowships						
F32's	-					
Research Career Development Awards						
K04's	1	35,700	-			
Other (R21, R23)	8	323,262	3	38%	-	
Total	61	4,102,023	29	48%	9	15%

*This table includes those grants which have already been funded and those which have been approved and will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for August 10 mailing to the Council Working Group.

TABLE 2

Approved NIA Competing Research Project Applications for FY 79* by Normalized Score

Biophysiology and Pathobiology Aging Program

Nutrition and Metabolism

Normalized Score	Number of Applications	Percent of Total
100-125		
126-150	1	4%
151-175	-	
176-200	1	4%
201-225	1	4%
226-250	-	
251-275	7	25%
276-300	6	21%
301-350	8	28%
351-500	4	14%
Total	28	100%

* This table includes those grants which have already been funded and those which will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for the August 10 mailing to the Council Working Group.

ENDOCRINOLOGY SUBPROGRAM OVERVIEW:

Investigations in endocrinology have provided remarkable and far-reaching advances in biomedical research. They have contributed to increased knowledge on; basic biological processes, prevention of diseases, improved health and quality of medical care. The importance of these studies are underlined by the fact that hormones, such as insulin and adrenocortical are absolutely critical for human survival. In fact, survival of the human species is not possible without a number of the major hormones, including those of the anterior pituitary, testicular, ovarian, placental, and thyroid hormones. Normal growth, development, and maturation cannot occur in the absence of growth hormone, adrenal steroids, parathyroid, gonadal hormones, insulin and glucagon. The personality and the social status of normal men and women have their bases in hormonally determined physical traits. Mental development is dependent on endocrine function and hormonal interactions. There is a fine hormonal interplay of endocrine function necessary for normal development. Later-occurring diseases and disabilities, such as hypertension, cancer, atherosclerosis, and benign prostatic hypertrophy seen in humans are hormone related and probably age related.

Clearly, the health and well-being of individuals at all ages are dependent on an intact, functioning endocrine system. Failure of secretion or even subtle changes in hormone titers can produce profound alterations of body function. For example, alteration of neurohormones can affect heart and brain function; decreased estrogen secretion can affect the integrity of bone, mucous membrane and skin; diminished testosterone secretion may affect muscle strength, libido, and sexual performance.

Selected Research Progress Summaries:

The overall objective of Dr. Wexler's research project is to determine whether hyperactivity of the adrenal glands, e.g., as a result of stress, Cushing's syndrome, is responsible for abnormal body metabolism which favor the development of degenerative changes resulting in premature cardiovascular disease and accelerated aging. This research has gathered evidence which would argue in favor of this contention.

The fundamental tenet in Dr. Wexler's experimental studies is that stress, e.g., the tempo of modern living, as well as physical stress, can alter internal body chemistry and condition organs and tissues toward premature vascular disease and aging. That is, aging is a disease - it can be accelerated (as in progeria) or retarded. Perhaps the intensities and ever-increasing stressful demands of modern living may contribute to accelerated aging through overactivity of the hypothalamic-pituitary-adrenal axis.

The findings he reports, reinforces his basic contention that overactivity the hypothalamic-pituitary-adrenal-gonadal axis on a chronic basis will eventuate in a spectrum of metabolic and pathophysiologic changes in which condition the body toward premature vascular disease concomitant with premature aging. At the same time, it underscores this contention that the process of accelerated aging greatly compromises the ability of the organism to cope with disease. Age, in calendar years, may mask underlying accelerated aging and, consequently, a middle-aged individual may have the "resistance" of an oldster in terms of hypothalamic-pituitary-adrenal function.

One of Dr. Wexler's projects, which was recently completed, involved the investigation of spontaneously hypertensive rats from weaning to old age. Paradoxically, despite their fulminating high blood pressure, these animals lived longer than normotensive rats, i.e., 33 months. Male and female SHR rats were autopsied at select time intervals from weaning to 28 months. Their blood pressure began to rise steeply at 4 to 5 weeks, reaching a zenith of 180 to 240 mmHg after four months. Elevated blood pressures were maintained in both sexes. After 20 months, the male SHR rats began to die of myocardial infarction and hypotensive crisis. Heart and adrenal gland wt. increased progressively, not only during the phase of rapidly rising blood pressure, but also during the period of plateaued but sustained high blood pressure. Radioimmunoassay of plasma levels of aldosterone, deoxycorticosterone, corticosterone, and prolactin, under both quiescent and mildly stressful conditions, demonstrated that the pituitary-adrenal axis of SHR rats increases progressively its propensity to respond to stress with maturation. This capacity to respond to stress was maintained despite the attainment of relative old age, i.e., two years, and despite severe high blood pressure. An incremental change in circulating prolactin, corticosterone, and aldosterone, as early as two months of age when blood pressure levels are beginning to rise, suggests that there may be some connection between the genetically programmed pathogenesis of the spontaneous hypertension and the progressively increasing (with age) sensitivity of the pituitary-adrenal axis to stress. Some of these animals developed nonfatal "strokes" and their high blood pressure became abnormally low.

Another highlight of Dr. Wexler's results pertains to the pathophysiologic differences between young versus old rats during an acute myocardial infarction. Young (90 days) and old (15 months) male Sprague-Dawley rats were subjected to an acute and massive myocardial infarct by giving them two injections of a large dose of isoproterenol. The animals were autopsied at sequential time intervals to ascertain the similarities or dissimilarities in the pathophysiologic events which attend acute myocardial infarction and repair in young versus old rats. Although the signs and severity of hypotensive shock appeared to be equal, mortality was higher in the old rats, especially during the acute necrosis phase. The older rats also manifested more severe and persistent congestive heart failure, i.e., hydrothorax. Serum enzymes (CPK, SGOT, SGPT and LDH), lipids (triglycerides, free fatty acids and cholesterol), glucose, and BUN levels manifested a dynamic rise and fall concomitant with the induced myocardial necrosis and repair phases with distinct differences in these metabolic changes between young and old rats. Despite initially higher circulating levels of corticosterone in the old versus young rats, the older animals manifested little or no increase in circulating corticosterone levels during the acute stress of myocradial infarction. This apparent lack of adrenocortical responsiveness was accentuated by the concomitant finding of greatly hypertrophied, hemorrhagic, and lipid-depleted adrenal glands in the old rats versus a dynamic increase in circulating corticosterone levels and alterations in the weight of adrenal and thymus glands of the young rats. During the myocardial repair phase, the young rats manifested extensive endocardial fibrosis, whereas the old rats displayed little or no endocardial fibrosis but copious and persistent myocardial edema and ground substance in keeping with their higher concentration of cardiac hexosamine. The pathophysiologic course of events which attends myocardial necrosis and repair is quite different in young versus old rats and may be related to the degree of responsiveness of the pituitary-adrenal axis which changes with age.

The objective of Dr. Longcope's project is to determine the contribution of the ovaries of postmenopausal women to the circulating levels of androgens and estrogens.

The results of this study indicate that in the majority of postmenopausal women the ovary functions only transiently, if at all, and that when functioning it normally secretes androgens and not estrogens. In no subjects could an ovarian gradient for estrone or dehydroepiandrosterone sulfate be found.

These are significant findings showing that ovarian function occurs in only a few postmenopausal women; that peripheral androgen conversion is the source of estrogens; and that the adrenal is the usual source for the androgens.

The studies conducted by Dr. Meites have attempted to elucidate the changes that occur in the hypothalamus, pituitary, gonads and reproductive tract of aging male and female rats in order to elucidate the mechanisms responsible for decline of reproductive and other endocrine functions.

Since Dr. Meites' results indicate that the ovaries of old rats are capable of near normal function under appropriate gonadotropic stimulation, it is concluded that the major cause for cessation of regular estrous cycles in old rats lies in altered hypothalamo-pituitary function.

In another study, the steady state concentration and turnover index (TI) of norepinephrine (NE) and dopamine (DA) were determined in the anterior and posterior hypothalamus of intact 18- to 20-month old constant estrous (CE) and of 4- to 5-month old proestrous and estrous rats, and in similar old and young ovariectromized rats.

In CE rats, concentration and TI of NE were significantly lower in the anterior hypothalamus as compared to young proestrous and estrous rats, whereas concentration and TI of DA were about the same in old and young rats. Ten days after ovariectomy, DA concentration in the anterior hypothalamus was increased similarly in both young and old rats, but NE levels were not altered in either age group. Both DA and NE TI were increased significantly after ovariectomy in the anterior hypothalamus of young, but not of old rats. Concentration and TI of NE and DA were not different in the posterior hypothalamus between old and young rats, and were not altered by ovariectomy. By ten days after ovariectomy, serum levels of LH and FSH in old rats were about half of those in young rats. These results indicate that in old CE rats, the decrease in NE and DA concentration and TI in the anterior hypothalamus is associated with a reduction in LH and FSH release and loss of cycling.

Dr. Cooper also has been investigating the mechanisms which contribute to age-dependent changes in the brain-pituitary-ovarian axis as well as the behavioral consequences of these changes.

The results indicate that regular ovarian function (when it ceased to cycle) can be reinstated when females are fed an L-tyrosine enriched diet and that this same diet, when introduced relatively early in the female's life, can prolong regular ovarian function.

These findings have led to the hypothesis that the disruption of regular ovarian function in the aging female is due primarily to age-related changes in CNS CA function. While this may indeed be the case, the fact that these treatments also influence CNS serotonin (5-HT) metabolism must also be considered. Sinemet, the agent found most effective in reinstating ovarian cycles, not only leads to a greater elevation of CNS CA, but also a greater suppression of CNS 5-HT when compared to L-dopa of L-tyrosine. Presumably, the influence on CNS 5-HT is mediated through the competition of L-dopa or L-tyrosine with the 5-HT precursor amino acid, L-typtophan at the level of the L-amino acid transport system into the brain.

Recent Applications and Funding Data:

Table I
Reviewed, Approved, and Funded NIA Competing Applications for FY 79* by Funding Mechanism

Biophysiology and Pathobiology Aging Program

Endocrinology

Funding Mechanism	Total Applications		Applications Approved by IRG		Funded by NIA	
	Number	Amount	Number	Percent	Number	Percent
Research Grants						
R01's	30	1,739,395	21	70%	9	30%
P01's	2	696,036	1	50%	1	50%
Research Training Grants						
T32's	-					
Fellowships						
F32's	4	58,000	3	75%	-	
Research Career Development Awards						
K04's	1	29,797	1	100%	-	
Other (R21, R23)	10	297,959	1	90%	4	40%
Total	47	2,821,187	35	74%	14	30%

*This table includes those grants which have already been funded and those which have been approved and will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for August 10 mailing to the Council Working Group.

TABLE 2

Approved NIA Competing Research Project Applications for FY 79* by Normalized Score

Biophysiology and Pathobiology Aging Program

Endocrinology

Normalized Score	Number of Applications	Percent of Total
100-125	-	
126-150	-	
151-175	1	3%
176-200	1	3%
201-225	1	3%
226-250	6	20%
251-275	8	28%
276-300	3	10%
301-350	6	20%
351-500	4	13%
Total	30	100%

* This table includes those grants which have already been funded and those which will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for the August 10 mailing to the Council Working Group.

EXERCISE AND PHYSIOLOGY SUBPROGRAM OVERVIEW:

Among all age groups, musculoskeletal disabilities are the primary cause for limiting the quality of life, rank second only to circulatory diseases in health costs, and third in frequency of occurrence among acute conditions. Also, musculoskeletal disabilities rank second in number of visits in hospitals, and third in number of operations in hospitals.

Impaired functions of the musculoskeletal system, however, have their greatest impact on the elderly, accounting for 20 percent of Medicare hospitalization costs. For example, clinical evidence and osteoporosis is present in almost all women over 75 years old. Since musculoskeletal disabilities are so prevalent in the elderly, an expanded research program on the musculoskeletal system, addressing exercise physiology and orthopaedics research, can effectively improve the quality of life for the fastest growing segment of our population at the least cost to benefit ratio for the public.

A program of research on function of the musculoskeletal system in the elderly can properly address the areas of prevention, diagnosis, treatment and rehabilitation.

Selected Research Progress Summaries:

Dr. Holloszy and his associates have been testing the hypothesis that exercise may exert beneficial effects on functional capacity and longevity (a) by protecting against obesity, loss of lean tissue, mineral loss from bone, hyperinsulinemia and glucose intolerance, which can all contribute to the increased morbidity and mortality, and the deterioraation in function that occur with aging, and (b) by maintaining the structural and functional integrity of the heart, skeletal muscles, autonomic nervous system, bones, and neuro-endocrine system, all of which deteriorate with physical inactivity. There is some evidence that a number of the adaptations to exercise run counter to the changes seen with aging.

On the basis of the limited information available, there is reason to hope that exercise could play an important role in Preventive Medicine by maintaining function and preventing physical disability in middle-aged and elderly individuals. There are approximately 23 million individuals 65 years of age or older in our population. Confinement to bed for acute and chronic illness averages 1411 days per 100 persons per year in the over 65 year age group compared with 650 days per 100 persons per year for the entire population. Furthermore, 43% of individuals above age 65 years have health determined limitations in activity, and 16% are unable to perform major acitvities because of chronic physical disabilities; the comparable figures are 12.7% and 3% for all age groups. In view of this high incidence of disease and disability, any beneficial effect of exercise in preventing physical disability and limitation of function in the older age groups would have a major impact. However, according to the "Rate of living - rate of O consumption" theory, which has never been disproved, exercise could accelerate aging and shorten the life span. Therefore, before exercise can become a scientifically acceptable preventive and therapeutic modality which can be confidently recommended for its health benefits, it seems essential to determine the effects of exercise on aging and longevity, and to obtain a better understanding of the long-term adaptations to exercise.

As part of Dr. Holloszy's project, Drs. Fell and Terblanche have been studying (a) the acute and chronic effects of exercise on glucose transport into muscle, and (b) the effects of lactate on glucose uptake and glycogenolysis in resting and contracting muscles. In addition, Fell is winding up a study that he and Dr. McLane conducted on the effects of iron deficiency on the O uptake capacity and endurance of contracting muscle. These studies are all being conducted on the perfused rat hindquarter preparation. The perfused hindquarter is one of the major experimental models that they plan to employ to evaluate skeletal muscle oxygen uptake capacity, endurance and contractile properties in the animals are providing the experience, and maintaining the skills, needed for the successful use of this preparation.

Dr. Newman, also a member of the team, is studying the effects of exercise-training on the sensitivity of the heart to catecholamines. In the process, he is setting up the isolated, blood perfused, isovolumic rat heart preparation, which he will later also use to evaluate the contractile function of the hearts from the aging animals.

Dr. Craig, another member of Dr. Holloszy's group, is studying the effects of exercise-training on insulin binding to, and insulin sensitivity of, rat adipocytes and epitrochleris muscles. He will later apply the same methodology to evaluate the effects of aging, and of the effects of exercise in aging animals, on insulin binding and insulin sensitivity of adipose tissue and skeletal muscle.

The primary objective of Dr. Lipton's research program is to learn how aging affects the central controls of body temperature. The main aim is to obtain information from experiments on laboratory animals which can be used both as a basis for understanding clinical problems in aged man and as a model for understanding changes in autonomic control systems in the aging brain. Specific aims are: (1) to learn whether there are changes in central sensitivity to naturally occurring biological constituents in the brain, to pyrogens and to hypothermogenic and hyperthermogenic drugs commonly taken by elderly patients (2) to determine whether the amount of recovery of thermoregulatory capacity after destruction of central temperature controls is reduced in aged animals.

The data collected by Dr. Lipton so far has several important implications for thermal physiology: (1) pyrogenic response in aged homeotherms of the sexes, (2) the effects on thermoregulation in aged homeotherms of drugs commonly prescribed for elderly patients. Appropriate interpretation of fever, a common sign of disease, is of considerable importance in treatment of patients of all ages. He has found that aged rabbits and squirrel monkeys, especially females, show reduced febrile response to pyrogens. At least a portion of this deficiency in febrile response is due to a reduction in central sensitivity to pyrogen. The recognition of the reduction in febrile response is important because body temperature in aged homeotherms is relatively low and small febrile reactions may be misinterpreted as normal temperature in diagnosis.

It is well documented that older patients are susceptible to accidental hypothermia and heat-stroke. An altered central sensitivity to drugs may contribute to dysthermia. He has found that old rabbits are especially sensitive to the hypothermogenic effects of chlorpromazine, a commonly prescribed tranquilizer. Further, the results indicate that in a cold environment the old animals are particularly susceptible to the central action of the drug. Findings such as these are useful in pointing up the potential for disastrous effects on body temperature of certain drugs when given to aged patients.

Recent Applications and Funding Data:

Table I
Reviewed, Approved, and Funded NIA Competing Applications for FY 79* by Funding Mechanism

Biophysiology and Pathobiology Aging Program Exercise

Funding Mechanism	Total Applications		Applications Approved by IRG		Funded by NIA	
	Number	Amount	Number	Percent	Number	Percent
Research Grants R01's	17	1,098,081	15	88%	6	35%
P01's	-					
Research Training Grants T32's	2	185,080	2	100%	1	50%
Fellowships F32's	1	14,500	1	100%	-	
Research Career Development Awards K04's	-					
Other (R21, R23)	5	180,157	3	60%	1	20%
Total	25	1,477,818	21	84%	8	32%

*This table includes those grants which have already been funded and those which have been approved and will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for August 10 mailing to the Council Working Group.

Recent Applications and Funding Data:

Table I
Reviewed, Approved, and Funded NIA Competing Applications for FY 79* by Funding Mechanism

Biophysiology and Pathobiology Aging Program

Physiology

Funding Mechanism	Total Applications		Applications Approved by IRG		Funded by NIA	
	Number	Amount	Number	Percent	Number	Percent
Research Grants						
R01's	8	615,200	6	75%	1	13%
P01's	-					
Research Training Grants T32's	-					
Fellowships F32's	-					
Research Career Development Awards K04's	-					
Other (R21, R23)	5	244,694	3	60%	2	40%
Total	13	859,894	9	69%	3	23%

*This table includes those grants which have already been funded and those which have been approved and will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for August 10 mailing to the Council Working Group.

TABLE 2

Approved NIA Competing Research Project Applications for FY 79* by Normalized Score

Biophysiology and Pathobiology Aging Program

Physiology

Normalized Score	Number of Applications	Percent of Total
100-125	-	
126-150	-	
151-175	-	
176-200	1	14%
201-225	-	
226-250	1	14%
251-275	1	14%
276-300	3	44%
301-350	1	14%
351-500	-	
Total	7	100%

* This table includes those grants which have already been funded and those which will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for the August 10 mailing to the Council Working Group.

TABLE 2

Approved NIA Competing Research Project Applications for FY 79* by Normalized Score

Biophysiology and Pathobiology Aging Program

Exercise

Normalized Score	Number of Applications	Percent of Total
100-125	-	
126-150	-	
151-175	-	
176-200	1	6%
201-225	1	6%
226-250	3	17%
251-275	2	10%
276-300	3	17%
301-350	3	17%
351-500	5	27%
Total	18	100%

* This table includes those grants which have already been funded and those which will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for the August 10 mailing to the Council Working Group.

ANIMAL MODELS AND RESOURCES SUBPROGRAM:

The Animal Models Subprogram was developed in 1974, from a single pilot study contract for $3,750, to rear and maintain Fischer 344 rats suitable for aging research. At the present, this subprogram has four contracts totaling over one million dollars. The contract specifications allow for the acquisition, rearing, maintenance and shipping of these animals to grantees of the NIA. The animals are maintained on a defined diet and specific pathogen-free (SPF) conditions. The rat colonies maintained under contract have been well characterized and this information has been made available to investigators through publications. At the present, characterization data are being gathered on the mouse strains maintained by contract.

Selected Contract Progress Summaries:

Contract Number:	N01-AG-4-2811
Contract Title:	Supply of Mice
Contractor:	Charles River Breeding Laboratories, Inc.
FY 79 Funding:	$42,000.00

Progress Summary: This contract was primarily undertaken to develop a readily available resource of aged, genetically defined and characterized strains of laboratory mice reared in a controlled environment. To meet this need, a colony of inbred strains, C57BL/6, BALB/c, and the inbred F-1 hybrid of the two inbred strains (CBF-1), was established in a specific pathogen-free (SPF) barrier enclosure, through the NIA contracting mechanism. Characterization data are under acquisition and will be available for distribution shortly. Information concerning rodent usage as an animal model is contained in a recent publication.

The colony census totals around 21,000 animals from 1 to 30 months of age. Currently, 850 one-month old C57BL/6 mice (800 males and 50 females), 250 one-month old male CBF-1 and 250 one-month old male BALB/c mice are added on a per-month basis, totaling 1,350 per month. Pathology monitoring is accomplished on a schedule as part of the contract specifications.

Proposed Course: This contract was scheduled to expire on May 16, 1979. On that date, for administrative purposes, it was scheduled to be merged with N01-AG-7-2128. It was extended one month and a half for additional time to accomplish this merger. The contract expired on June 30, 1979.

Contract Number: N01-AG-3-2725

Contract Title: Maintenance of a Long-term Aged Rat Colony

Contractor: Charles River Breeding Laboratories, Inc.

FY 79 Funding: $457,000.00

Progress Summary: This contract provides Fischer 344 male, female and male retired breeder rats suitable for research on aging to investigators funded by the NIA. They are acquired, maintained and shipped under contract specifications. Animals are reared on a defined diet under specific pathogen-free (SPF) conditions. Animals have been characterized and this information has been published. Survival is 95 percent, while maximum life span is 30 months.

The colony census totals around 18,000 animals from 1- to 30-months of age. Currently, 650 one-month old virgin males, 75 one-month old virgin females, and 150 nine-month old retired breeder males are added on a monthly basis, totaling 850 animals per month. Pathology monitoring continues on an intermittent basis. Colony size must be increased to meet the continually growing demands for well-characterized research animals.

A Justification for Noncompetitive Procurement (JNCP) is valid until May 1981. The contract expires June 1980.

Proposed Course: This contract will be supported until June 1980. A Sources Sought will be issued within six months to determine which of the following would become a feasible option for further support; 1) have the colony supported by the contractor without government funds; 2) convert the current R&D contract to a supply contract or 3) recompete based on the sole source test of the suppliers. Even if recompetition were the chosen course, the value of many ongoing studies could be severely compromised. A new contractor will require 2 to 3 years to build up an adequate resource. In this period, two colonies, the old and the new one, have to be maintained which would be extremely costly. Preparation for recompetition of the contract should begin about August/September 1979.

Contract Number: N01-AG-6-2135

Contract Title: Laboratory Rat Pilot Studies on Inbred Strains and Selected Hybrid Crosses (In a Defined Environment)

Contractor: Charles River Breeding Laboratories, Inc.

FY 79 Funding: $86,400

Progress Summary: This contract was begun in June 1976, to: (1) develop a rat model for aging research that provides a broad gene pool with maximum genetic control; (2) develop an F-1 hybrid rat strain free of pathological conditions seen in currently available inbred strains and outbred stocks; (3) develop a rat strain which has much of the generalization to aging as an outbred stock with the uniformity and predictability of pathological and biological characteristics of the inbred strains. Four strains were chosen for development as inbred and outbred strains: the Fischer 344, Brown Norway, Lewis and Buffalo. Characterizations data are being collected and will be available on the strain selected for final development.

The start of this project was delayed several months while a fecund and fertile Brown Norway was acquired. The project is proceeding in accordance with specifications set forth in the contract. A no-cost extension for a one-year period beyond the June 30, 1979 expiration date was requested on May 3, 1979.

All animals scheduled for input into this contract have been set up. Monitoring for characterization data, body weight, and other pathological lesions will continue throughout the contract life. Plans should be made for the selection of the F-1 hybrid to be developed on subsequent contract.

Contract Number: N01-AG-6-2135

Contract Title: Laboratory Rat Pilot Studies on Inbred Strains and Selected Hybrid Crosses (In a Defined Environment)

Contractor: Charles River Breeding Laboratories, Inc.

FY 79 Funding: $88,400

Progress Summary: This contract was begun in June 1976, to: (1) develop a rat model for aging research that provides a broad gene pool with maximum genetic control; (2) develop an F-1 hybrid rat strain free of pathological conditions seen in currently available inbred strains and outbred stocks; (3) develop a rat strain which has much of the generalization to aging as an outbred stock with the uniformity and predictability of pathological and biological characteristics of the inbred strains. Four strains were chosen for development as inbred and outbred strains: the Fischer 344, Brown Norway, Lewis and Buffalo. Characterizations data are being collected and will be available on the strain selected for final development.

The start of this project was delayed several months while a fecund and fertile Brown Norway was acquired. The project is proceeding in accordance with specifications set forth in the contract. A no-cost extension for a one-year period beyond the June 30, 1979 expiration date was requested on May 3, 1979.

All animals scheduled for input into this contract have been set up. Monitoring for characterization data, body weight, and other pathological lesions will continue throughout the contract life. Plans should be made for the selection of the F-1 hybrid to be developed on subsequent contract.

Contract Number: N01-AG-6-2136

Contract Title: Barrier-reared and Maintained, Aged Sprague-Dawley Derived Rats

Contractor: Harlan Industries, Inc.

FY 79 Funding: $135,958

Progress Summary: This contract, originally began in June 1976, was to provide another commonly used rat strain (the Fischer 344 rat is an inappropriate model for some aging research), reared on a defined diet in a pathogen-free environment. This would provide an additional resource to meet demand for the laboratory rat. The contract was for a five-year period, funded in five annual increments. By the end of the second year, it became apparent that the colony was not progressing as anticipated. There were unusually high rates of mortality and morbidity in the older age groups, rendering the colony a less than optimum resource for aging research. Prior to the third-year increment, it was decided to decrease the monthly input the colony and increase the monitoring of the pathology reports to acquire additional data. On the basis of the pathology reports, it became evident that the strain of rat itself was the problem and not environmental factors. Since the rat strain was provided to the contractor by the government, a termination for the convenience of the government was initiated which became effective on March 31, 1979. Prior to the termination date, approximately 1,000 animals of various ages were distributed to investigators for pilot studies.

Other Developments: In spite of the termination of this contract, the need for a second rat strain for use in aging research remains. The termination of this contract has caused hardships for some investigators and has forced others to change to the Fischer 344 colony, which is in demand already. To compensate for the loss of this colony of rats, the size of the existing colony maintained under contract N01-AG-7-2127 was increased. See details in that contract report.

Contract Number: N01-AG-7-2118

Contract Title: Survey of Animal Models for Research on Aging

Contractor: National Academy of Sciences, National Research Council, Institute of Laboratory Animal Resources

FY 79 Funding: $99,400

Progress Summary: This contract began in September 1977. It was designed to acquire, evaluate, and interpret data and make information available on the basic criteria necessary for the selection of vertebrate models to study the aging processes. At the present, selection criteria does not exists to guide investigators in choosing an appropriate animal models. The available information available is scattered throughout the scientific literature, unpublished laboratory records and personal experiences of investigators holding considerable expertise in the use of a particular animal model in aging research.

After the initial identification of the animals to be studied, namely, rats, mice, nonhuman primates, and lagomorphs, work began on the collection of the available information. The subcommittees have met and sorted all the relevant information, in view of the amount of the material in the final report but will be saved on microfiche. This contract represents the first major effort at gathering and organizing this type of information. All subcommittee reports are completed, and a no-cost, one-year extension has been requested to complete the work in preparation for publication.

Contract Number: N01-AG-7-2127

Contract Title: Acquisition, Maintenance, and Distribution of a Colony of Barrier-reared, Aged, Retired Breeder Sprague-Dawley Rats

Contractor: Charles River Breeding Laboratories, Inc.

FY 79 Funding: $100,000

Progress Summary: This contract, awarded in September 1977, was primarily designed to provide a resource of a commonly used laboratory rat which could be used for comparison and evaluation of intra- and interspecies data. Prior to the acquisition of this colony, the Fischer 344, was the rat principally used in aging research. The study of aging processes requires availability of a variety of species and strains of animals in large enough quantity to accommodate the ever-increasing demand for well-defined research animals.

A standing colony of 811 animals, ages 6 to 30 months, currently exists. To this colony, 50 six-month old retired breeders are added montly. This colony is currently being expanded to include input of 200 six-month old male retired breeders. This contract expires on March 31, 1980. Recompetition plans should be submitted to Research Contracts Branch/NIH no later than September 1979, to avoid interruption of the availability of this commonly used stock.

On October 30, 1978 a Sources Sought Announcement, seeking a standing colony of about 4,500 mature and aging rats (250 pathogen-free retired breeder male Sprague-Dawley rats per month) was issued. Only sources who could demonstrate a stock or sub-stock with a mean survival over 75% at 24 months, as well as personnel, equipment and facilities to maintain the size of the colony requested, needed to reply. This was followed by a second announcement naming the current contractor as the only known source to meet this request and seeking any other sources who could respond with confirmatory data. The findings of the Sources Sought provided for the increase in the size of the existing contract described above (N01-AG-7-2127).

Contract Number: N01-AG-7-2128

Contract Title: Development of a Colony of Multigenotypic Aged Mouse Strains

Contractor: Charles River Breeding Laboratories, Inc.

FY 79 Funding: $727,847

Program Summary: This contract was begun on September 30, 1977, to develop, define, maintain and distribute commonly used strains of laboratory mice necessary to provide model systems for research in the ever-increasing fields of neuroscience, pharmacology, nutrition, genetics, and psychology. The availability of a wide variety of strains of laboratory animals is important to the development of any program of studies of comparative aging, such as those supported by the NIA. To facilitate these programmatic initiatives of the NIA, it is essential to have available a well characterized and genetically defined animal model. This contract will provide a resource of varied strains of mice for comparative studies on different aspects of the aging process.

At the present, the BALB/c, C57BL/6, NUDES (athymic), DBA/2N, B6D2F1, B6C3F1, 129 and A/HeJ strains are being entered into a production colony at a rate determined by the contract. The following strains will be added within the next fiscal year: CBA/HT6, CBA/ca, CFW (Webster), and CBF-1.

As indicated elsewhere, for administrative purposes, this contract was merged with the expired contract N01-AG-4-2811 on June 30, 1979. Animals will be continually provided from the new portion of this contract while standing colonies of the designated strains are being built up.

Other Major Developments:

1. Computerization of the projection analysis program is completed except for retired breeder colony. Inventory program being developed and data sets completed.

2. Publications:

   a) "Pathological Changes During Aging in Barrier-reared Fischer 344 Male Rats," Coleman, Gerald L., *et al*, Journal of *Gerontology*, 1977, Vol. 32, No. 3, 258-278.

   b) "Development of the Rodent as a Model System of Aging," Book II, Gibson, Don C., Adelman, R.C. Finch, C., Eds., U. S. Government Printing Office: DHEW Publication No. (NIH) 79-161.

3. Compared to other commonly used species of animals, the nonhuman primate has had a limited use in aging research. The pig-tailed macaque (*Macaca nemestrina*) is the only species which has been the subject of aging studies to any significant extent. The available information on the nonhuman primates is limited but what little is known indicates that, in many respects, the aging process in nonhuman primates is similar to that in man. The availability of these animals, in significant numbers, for aging research is an important recent development. A literature search conducted by the subcommittee of the Institute of Laboratory Animal Resources contract shows about 80 percent of published reports on aging in nonhuman primates have appeared since 1970. The maintenance of large colonies of nonhuman primates, similar to the rodent colonies, would be a complex and expensive enterprise.

4. A cooperative use of existing colonies at various universities and primate centers throughout the country is being planned. To this end, a census survey was initiated and completed, which was reported in the 1978 BPAP program review.

   a) Several other cooperative endeavors have been initiated. Preliminary discussions with Animal Resources Branch/DRR have resulted in a cooperative effort to identify aged nonhuman primates (15 years and older) at various primate centers. This joint effort was aimed at identifying and selecting animals for research based on a defined criterion of animal characterization and health status. This effort can be further expanded to identify investigator needs, matching these with colony census so that a more cost-effective use can be made of this limited resource.

   b) Recently, discussions have been held with NINCDS concerning their interest in a cooperative endeavor using nonhuman primates (*Macaca mulatta*) to study senile dementia and CNS aging (See Appendix III for further details).

5. On September 6, 1976, the NIA and the Veterans Administration (VA) entered into an interagency agreement to furnish animals from NIA contract supported colonies to VA approved and funded aging research projects. Information and instructions for the acquisition of NIA animals are published in a yearly VA circular.

The NIA continues to support a large number of VA projects through both direct purchase and pilot study acquisitions. The NIA has supplied 13 projects with over 2,384 animals during the period of March 1976 to the present. Nine of these projects have received animals on a pilot study basis. VA projects reimburse the NIA contracts at per diem cost. Pilot study animals are supplied for the cost of shipping and crates only. Copies of final reports and articles for publications identifying NIA as the supplier of animals must be furnished to the NIA.

Recent Applications and Funding Data:

Table I
Reviewed, Approved, and Funded NIA Competing Applications for FY 79* by Funding Mechanism

Biophysiology and Pathobiology Aging Program

Pathobiology and Animal Models

Funding Mechanism	Total Applications		Applications Approved by IRG		Funded by NIA	
	Number	Amount	Number	Percent	Number	Percent
Research Grants R01's	8	408,467	4	50%	1	13%
P01's	-					
Research Training Grants T32's	-					
Fellowships F32's	-					
Research Career Development Awards K04's						
Other (R21, R23)	2	113,772	1	50%	1	50%
Total	10	522,239	5	50%	2	20%

*This table includes those grants which have already been funded and those which have been approved and will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for August 10 mailing to the Council Working Group.

TABLE 2

Approved NIA Competing Research Project Applications for FY 79* by Normalized Score

Biophysiology and Pathobiology Aging Program

Pathobiology and Animal Models

Normalized Score	Number of Applications	Percent of Total
100-125	-	
126-150	-	
151-175	-	
176-200	1	20%
201-225	-	
226-250	2	40%
251-275	-	
276-300	1	20%
301-350	-	
351-500	1	20%
Total	5	100%

* This table includes those grants which have already been funded and those which will probably be funded before the end of this fiscal year. Preliminary August Council data will be added for the August 10 mailing to the Council Working Group.

NIA ANNUAL REPORT

Report of Epidemiology, Demography, and Biometry Program

Page

1. Overview of NIA's Epidemiology, Demography, and Biometry Program EDBP-1

2. Population and Clinical Research Analysis Group EDBP-5

3. Population Dynamics and Research Analysis Group EDBP-21

NIA ANNUAL REPORT
OCTOBER 1, 1978 THROUGH SEPTEMBER 30, 1979
EPIDEMIOLOGY, DEMOGRAPHY, AND BIOMETRY PROGRAM

On October 1, 1978, the EDBP received its first budget and commenced as an operating program. The overall mission which we are developing is to serve as the NIA focal point for quantitative population base research on health and disease in the aging including the broad disciplines of medicine, biostatistics, epidemiology, economics, sociology, and demography. As is predictable the first year was one of growth and development of new techniques and new territories. The professional staff consists of Jacob A. Brody, M.D., Epidemiologist (Associate Director, NIA), Clifford H. Patrick, Ph.D., Research Demographer, Joan Cornoni-Huntley, Ph.D., Senior Research Epidemiologist (Expert Appointment), Douglas A. Parker, Ph.D., Sociologist (IPA to EDBP for 2 years commencing August 1979), Yuko Palesch, M.S., Research Statistician and Dennis Cosmatos, M.S., Research Statistician (joined EDBP in June 1979). Our budget for contracts and operating expenses was approximately $1,500,000.

Our research is conducted primarily by EDBP staff supplemented and augmented by research contracts and interagency agreements and numerous working agreements with other government and nongovernment organizations. These latter areas are developing rapidly and successfully and will be alluded to in this summary and in individual project reports. We have formulated a division of our projects into a Population and Clinical Research Analysis group and a Population Dynamics and Research Analysis group (this differs from last year's report). Our plan is to have these develop into separate Branches. The former is involved primarily with clinical applications of population research utilizing for the most part primary data sources. The latter involves demographic and economic research and relies primarily on secondary data analysis.

We have had several gratifying accomplishments in the area of population and clinical research and analysis coupled with some disappointments as we learn the difficulties of the logistics of implementing programs. Through an interagency agreement with the National Heart, Lung, and Blood Institute we have transferred funds to conduct a detailed objective quantification of disabilities in the Framingham population which subsequently we will relate to the known health status of the individuals. We worked most of the year writing and modifying a contract to develop one or more reference populations of the elderly for integrated studies of medical, socioeconomic, and behavioral aspects of health, "Establishment of Populations for Epidemiologic Studies." We hope to include general social and behavioral aspects as well as "soft medical signs" of the elderly such as constipation, dermatitis, sleep disorders, and various forms of chronic pain and discomfort. The final contract responses will be received by September 14, 1979, but the actual contracts will not be awarded until next fiscal year.

With nutrition as the focal issue, we have entered into an interagency agreement with the National Center for Health Statistics to conduct a follow-up study of the Health and Nutrition Examination Survey (HANES). NIA is the lead agency with expected support from NCI, NHLBI, and NIAAA. The cohort examined in HANES I will be followed up to determine patterns or morbidity and mortality and relate them to the data collected on the initial survey. A feasibility study will be completed this fiscal year and forms have been cleared through OMB.

We have had three joint meetings and a review session with NHLBI concerning a study of systolic hypertension in the elderly. At the present time some modification of the grant mechanism is comtemplated for a major clinical trial which will last 10 years and cost between $15 and $40 million dollars. It is possible that some funds will be committed during this fiscal year.

Senile dementia is a major thrust of EDBP and a special initiative of NIA. We are working closely with NIMH and NINCDS at many levels from basic laboratory procedures to field studies. We have joined in regular monthly meetings with the other two Institutes. We have also entered into an interagency agreement with NIMH to which NINCDS will probably contribute. NIMH has a contract with Yale University to conduct a survey of mental illness in the New Haven area designated an epidemiologic catchment area (ECA). We are supplementing this study by oversampling the population age 65 and over in order to give us a population in excess of 2,000 to determine prevalence of senile dementia and other detectable mental illnesses in the elderly. This will be the first step in a series of proposed laboratory and field studies to determine the incidence and prevalence, natural history and predisposing factors involved in the senile dementias.

We are developing studies of the epidemiology of the last days of life to develop realistic information for physicians and patients on the basic events associated with dying such as who dies peacefully in his sleep, who dies in great pain, who dies in the presence of his family, who dies after a long illness with full awareness of his impending` demise, and who dies suddenly with no warning.

We are further interested in hospitalization and use of pain medication. In order to find a suitable population for this study we have entered into negotiations with NCHS, HCFA, NCI and NHLBI. No study population has emerged as yet, but we are hopeful, particularly in collaboration with NHLBI, that a suitable population will be found.

With NCHS we have an interagency agreement and are developing data on the epidemiology of osteoporosis and its relation to hip fracture, the rate of hysterectomies and time trends, morbidity and mortality studies of accidents, and the relationship of morbidity from stroke to the striking decline in mortality from stroke.

In the area of Population Dynamics and Research Analysis, demography and economics are the central disciplines. Major efforts have been involved in developing both an overview and the ability to become a focus for statistical data on aging. In addition to a major conference referred to below several interagency agreements have been completed with the Bureau of the Census. These involve development of demographic characteristics of the elderly population from 1950 to 2050 and an indepth study of the characteristics of the elderly population in institutions. Through a collaborative agreement with Columbia University we are exploring the spending patterns of the elderly and its health implications by determining the proportion of incomes spent on health, housing, and other major goods and services. We have also issued a contract to develop the first macroeconomic model of the U.S. economy which includes age structure. We have also been working with the University of Pennsylvania on evaluation of accepted effective medical treatments. A great amount of time has been devoted to learning the existence and usefulness of the various data sets which pertain to the elderly in other Federal agencies as well as in the private sector. We are negotiating with HCFA to issue a joint contract relating to health cost containment. We are also completing the necessary negotiations and paperwork to develop several interagency agreements with the Social Security Administration to utilize data on their Retirement History Survey and on recently entitled beneficiaries. In addition we have been collaborating very closely with the Director of NIA in developing the composition and representation for the 1981 White House Conference on Aging.

The EDBP has held three major conferences during the year. The first in October 1978 was a planning meeting for Technical Consensus on Estrogen Use and Postmenopausal Women. In this we brought together approximately 20 scientists to discuss the advisability and the critical questions involved in holding a technical consensus exercise on this subject. The Technical Consensus on Estrogen Use and Postmenopausal Women will be held in September 1979. A panel of 10-15 experts and interested individuals will attempt to reach consensus in a large open meeting to which 200-500 individuals are expected to attend. The central issue is the benefits from the use of estrogen in controlling symptoms associated with menopause and possibly in preventing or arresting osteoporosis versus the increased incidence of endometrial carcinoma in women using estrogen. The third conference on "Demographic and Health Information for Aging Research: Resources and Needs" emphasized existent Federal data sets, their usefulness and limitations in research. This conference brought together approximately 150 interested people and opened perhaps a Pandora's box relating to data which has been collected and the uneven usefulness, availability, and exploitation of the information.

This has been a year of growth, struggle, and learning for use. We are becoming more sophisticated about the problems of contract, interagency agreements and the delicacies of OMB clearance of forms. We are learning to be more realistic concerning the time frame within which programs can be developed and initiated. We have a greater appreciation for the need for careful early planning and the reality of how time consuming intelligent planning is.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 02010-01 EDBP

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Follow-up of Health and Nutrition Examination Survey I (HANES I)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:
Joan Cornoni-Huntley, Ph.D.
Senior Research Epidemiologist
OTHERS:
Jacob A. Brody, M.D.
Associate Director

Dennis Cosmatos, M.S.(Hyg.)
Research Statistician

COOPERATING UNITS (if any)

National Center for Health Statistics, Division of Analysis

LAB/BRANCH
PCRA

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.20	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to design and complete a follow-up of persons examined in the HANES I to study how factors previously measured on these subjects relate to the health conditions that have developed since the survey. The three major areas for prediction of outcome are 1) nutrition 2) risk factors for chronic disease and 3) health care utilization. The survey will have a household interview including self-reporting of health conditions, utilization of health services and behavioral and social status plus some physical measurements as blood pressure, height and weight.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: During the years 1970 to 1975, the National Center for Health Statistics conducted the Health and Nutritional Examination Survey (HANES I). This was the first and probably the largest indepth national survey of health and nutrition ever conducted. A cohort of about 28,000 people were selected to be used for estimating the prevalance of various conditions and the distribution of physical, psychological, and biochemical characteristics for the entire U.S. population.

In order to maximize the utility of this baseline data, the individuals originally in the sample can be followed over time and the conditions which have developed since the initial survey can be identified. The ability to identify the antecedent and consequence relationships by this method is important in analyzing the factors related to problems of aging. Those factors that exist in one point in time are observed as predictors of health status at a later time.

Most of the problems associated with aging are a consequence of long-term and low dosage exposure to a combination of environmental, dietary, social, and demographic factors. By following this cohort over time, this study aims at identifying the levels and combinations of these factors that are associated with specific health conditions.

Methods Employed: The proposed full scale project is to complete a follow-up study on those individuals who were 25 years and older at the time of their participation in the initial HANES survey.

A feasibility study will be conducted in order to estimate the ability to find and interview the persons who had previously particpated in the survey, and to estimate the cooperation that can be expected in the follow-up survey from the respondents and their medical care providers.

A pretest will then be administered to those who participated in the HANES I pretest, in order to perfect and finalize the questions to be used on the actual HANES I Follow-up Survey.

Major Findings: Work in progress.

Significance to Biomedical Research and the Program of the Institute: The HANES I Follow-up Survey will provide national estimates of risk to certain health conditions for a representative sample of the United States population, and allow relationships to be drawn between these estimates and nutritional, social, demographic, and behavioral characteristics of the population at two points in time.

It is of primary importance for the National Institute on Aging to completely understand the epidemiology of the problems related to aging. The surveys conducted by the National Center for Health Statistics give baseline information on a representative sample of the U.S. population. This extensive amount of information can be used to identify the pertinent associations related to health outcomes of individuals in the older age groups. This analysis would act as a screening of data in the physical, psychological, social areas as well as the utilization of health care to identify and define appropriate hypotheses for further investigations. Through the information obtained by these follow-up studies, in-depth research can be designed to answer specific questions of interest to the National Institute on Aging.

Proposed Course: Reimbursable agreement continuing.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 02070-01 EDBP

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Review of the Current Literature and Surveys of Nutritional Factors Associated with Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:
Joan Cornoni-Huntley, Ph.D.
Senior Research Epidemiologist, EDBP, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
PCRA

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

For the planning and designing of future NIA research this project will review and evaluate the current literature on problems of nutrition of the elderly. In addition, nutritional survey research shall be reviewed in terms of sampling, content and methodology. The evidence of physiological factors related to or involved with nutritional problems will be examined. The factors will include dental problems, gastrointestinal functioning, social-psychological factors and drug use. This information will be organized into a useful reference document.

PROJECT DESCRIPTION:

Objectives: The current literature on problems of nutrition of the elderly is to be reviewed and evaluated. In addition, survey research on nutritional behavior and status of the elderly is to be reviewed for sampling, content, and methodology. We are interested in examining the evidence of physiological factors related to or involved with nutritional problems. For example, dental problems and gastrointestinal functioning are conditions that negatively effect nutritional status. Social-psychological and economic factors must also be considered. The use of drugs are believed to affect food intake. These areas should be reviewed and organized into a useful reference for planning and designing future NIA research projects.

Methods Employed: The project will be completed in three parts. The first part will include the literature review of nutritional studies of elderly populations. This section will focus on studies of gastrointestinal and dental problems and social-psychological factors.

The second part will review surveys including a critique of existing survey form, sample, and methodology.

Finally, a report will be produced summarizing existing information and recommending direction of future studies.

Major Findings: Work is in progress.

Significance to Biomedical Research and the Program of the Institute: A multitude of physiological, psychological, social and economic factors have been reported related to nutritional status and behavior of the elderly. Also several nutrition oriented surveys have been completed in the last 10 to 15 years of various population groups including different socio-economic levels and ethnic backgrounds. In initiating, planning and designing future research, a critical review of current literature and nutritional survey research would be beneficial.

Proposed Course: Contract to Columbia University continuing.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 02080-01 EDBP

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Secondary Analysis of HANES - Predictive Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:

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Senior Research Epidemiologist

OTHERS:

Dennis Cosmatos, M.S. (Hyg.)
Research Statistician

Frances Davidson (IPA)
Georgetown University
School of Medicine
Washington, D.C.

COOPERATING UNITS (if any)

National Center for Health Statistics

LAB/BRANCH

PCRA

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.10	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A variety of analyses are being completed on the data collected for the HANES I to identify associations existent in the cross-sectional information. These results will provide the basis for hypotheses to be investigated in the HANES I Follow-up Survey. Such studies include the relationship of nutritional behavior to health status. In addition, validity and reliability studies will be completed to evaluate self reporting of health conditions.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: In preparation for the extensive follow-up survey for HANES I preliminary analyses of the baseline data are being completed. Nutritional studies including dietary intake, food composition and biochemistries are being analyzed to describe the status of the population at the time of the original examination. Through these preliminary analyses subjects may be grouped into levels of nutritional intake and dietary behavior.

In addition validity and reliability studies are being completed to evaluate self-reported illness as compared to physical examination.

Methods Employed: Appropriate statistical methodology will be performed to identify homogeneous groups according to patterns of nutritional behavior. Differences in health conditions of these groups will be compared. Sensitivity and specificity measures will be calculated for validity studies. Other sophisticated statistical methods will be employed when necessary.

Major Findings: Work in Progress.

Significance to Biomedical Research and the Program of the Institute: The Follow-up Survey of HANES will provide prospective data on a representative sample of the U.S. population. The relationships of the original data collected in 1971-74 to outcome conditions will be studied to define predictive functions. To generate pertinent hypotheses, the results of these analyses should be obtained. Therefore, this project will enhance the value of the HANES Follow-up Survey.

Proposed Course: Continuing

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 02090-01 EDBP

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

General Analysis of National Center for Health Statistics Data Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:

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Research Statistician

Jennifer Madans, Ph.D.
NCHS

Joel Klienman, Ph.D.
NCHS

COOPERATING UNITS (if any)

National Center for Health Statistics, Division of Analysis

LAB/BRANCH
PCRA

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.30	.10	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This contract allows the National Institute on Aging to work cooperatively with the National Center for Health Statistics in conducting analyses of appropriate data sets as the need for such analyses arises. By working in this joint manner pertinent questions can be answered more efficiently and the level of research is likely to be of higher quality than if either institute worked alone.

At the present time the research topics include: 1) the relationships between the existance of flouride in drinking water and osteoporosis, 2) changes in incidence of stroke, 3) analysis of Family Data, 4) differences in lean body mass between the sexes and 5) mortality rates from accidents.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: The purpose of this project is to provide analysis of NCHS Data to answer specific hypotheses related to the elderly. The research problems being investigated are:

1. The relationship between osteoporosis and the existence of fluoride in drinking water
2. Estimate of the incidence of stroke by studying changes in reported paralysis or limited activity due to stroke
3. Family Studies
4. Lean Body Mass
5. Changes in the incidence of accidents

Methods Employed: The analyses will include a clear statement of the problem; identification of the appropriate data sets; determination of the best statistical methods for analysis; prepare work tapes; program and process the data analysis, interpret the results and generate further hypotheses.

Major Findings: Analysis in Progress

Significance to Biomedical Research and the Program of the Institute: Obtaining estimates on incidence, prevalence, change in these statistics and associations for the U.S. population about problems such as osteoporosis, stroke, accidents plus national estimates concerning social functions are a contribution to biomedical research.

Continually questions are generated and information is needed pertaining to problems of the aged. This funded mechanism to obtain answers from within the National Data System is extremely useful.

Proposed Course: Reimbursable Agreement - continuing

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 03000 EDBP

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Risks and Benefits of the decline in estrogen use by postmenopausal women.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:
Yuko Y. Palesch, Statistician, EDBP, NIA

OTHERS:

Jacob A. Brody, M.D.; Associate Director, EDBP, NIA
Arthur White, Grants Associate
NIH

COOPERATING UNITS (if any)

LAB/BRANCH
PCRA

SECTION
Epidemiology, Demography and Biometry

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Objectives/Issues addressed: Review the trends in estrogen use in the past decade by peri- and postmenopausal women. Discuss the current status of risks and benefits of estrogen use.
Approach/Methodology: Abstract data on estrogen drug appearance from National Disease and Therapeutic Index volumes (1970-1978). Mortality and incidence data of endometrial cancer for the U.S. is also collected. Review current literature on estrogen as risk or beneficial factor.
Major Findings: A drastic decline in estrogen drug appearances is observed during 1975-76 following the publication of several case-control studies linking estrogen use with endometrial cancer.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 04010-01 EDBP

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Catchment Area Study of Senile Dementia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:
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William Eaton, Ph.D.
NIMH

Ben Locke
NIMH

COOPERATING UNITS (if any)
National Institute of Mental Health
Center for Epidemiologic Studies

LAB/BRANCH
PCRA

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.05 (NIA)	.05 (NIA)	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In conjunction with the National Institute of Mental Health, NIA is completing a study of senile dementia in an adult community population. A contract has been awarded to Yale University to survey a sample of the New Haven, Connecticut adult population for this purpose. NIA is specifically interested in defining the occurrence of senile dementia in persons 65 years of age and older. The study will consist of a household interview and a follow-up of all subjects. A questionnaire has been developed by the investigators.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: The Division of Biometry and Epidemiology, National Institute of Mental Health (NIMH) is in the process of investigating the occurrence of mental illness in an adult community population. An award has been made to Yale University to survey a sample of the New Haven, Connecticut adult population for this purpose. The National Institute on Aging (NIA) is specifically interested in defining the occurrence of senile dementia in persons over 65 years of age. Since this requires a larger sample population, the study population that has been developed by Yale University for NIMH will be expanded. In the development of the descriptive epidemiology of the prevalence of senile dementia in a community, the NIA shall work with the staff of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS).

Methods Employed: In addition to the household sample of 3000 in the original ECA Research design for the New Haven site, persons 65 years of age and older shall be selected from households for interviews such that 2,000 additional persons 65 and over are included in the sample. The household sample as referred to throughout the design specifications will thus consist of 3,000 completed interviews representing the adult (over 18) population in the catchment area, and an additional 2,000 completed interviews representing the aged (65 and over).

A questionnaire developed by the investigators will be administered to all persons in the sample and a follow-up of each individual will be included in the study.

Major Findings: Work in progress.

Significance to Biomedical Research and the Program of the Institute: As more people age more people will develop senile dementia. Prevalence among those over 65 of age with severe progressive disease has been found in different studies to range from 1 to 9 percent and up to 15 or 20 percent for milder forms. No epidemiologic pattern for senile dementia has been discerned by race, sex, geographic, occupation, environmental or infectious exposures. This project will attempt to provide epidemiologic patterns for etiologic clues.

Proposed Course:

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 04011-01 EDBP

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Natural History and Progression of Senile Dementia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:

Joan Cornoni-Huntley, Ph.D.
Senior Research Epidemiologist, EDBP, NIA

OTHERS:

Jacob A. Brody, M.D.
Associate Director, EDBP, NIA

Elizabeth Parker, Ph.D.
NIAAA

COOPERATING UNITS (if any)

National Institute on Alcohol Abuse and Alcoholism

LAB/BRANCH

PCRA

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, Bethesda, MD

TOTAL MANYEARS: .10	PROFESSIONAL: .10	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Epidemiologic studies of senile dementia are needed in order to determine if the disease occurs in specific patterns by age, sex, race or other demographic and social characteristics and if such patterns would provide etiologic clues. This project will characterize persons within a community based, non-institutionalized sample who appear to have senile dementia and/or depression or to be at high risk of having these disorders. This sample will be compared with an unaffected group. Both samples will be followed over a three year period to discover risk factors and etiologic events.

PROJECT DESCRIPTION:

Objectives: This project will seek to determine if there are specific patterns or entities of senile dementia. An epidemiologic study of these conditions will be completed to define etiology and preventive measures.

Within a community-based non-institutionalized sample of persons felt to have depression and/or senile dementia or to be at high risk for having these disorders will be characterized and followed over three years. This sample will be compared with an unaffected group in order to discover risk factors and etiologic events.

Methods Employed: From a free living population of a defined community, a sample of persons will be identified as positive for senile dementia and depression by a screening instrument. These persons shall be evaluated by a complete clinical, indepth assessment. In addition, a representative sample of persons who appear to be normal or negative in regard to depression and/or senile dementia according to their response on the screening questionnaire. Both cases and controls will be followed for 3 years with contacts every 6 months to determine outcome of senile dementia and depression.

Major Findings: Work in progress.

Significance to Biomedical Research and the Program of the Institute: Little is known about the occurrence of senile dementia and depression in persons over 65 years of age. Etiologic mechanisms and risk factors are unknown; the natural history of these illnesses is obscure. Senile dementia and depression are apparently the most common disorders which primarily affect mental abilities in the elderly. Moreover, especially early in the illness, the two disorders may be indistinguishable. This project will attempt to provide a better definition of these illnesses and identify associated factors.

Proposed Course: Project being planned and designed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 04050-01 EDBP

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Natural History of Last Days of Life

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:

Jacob A. Brody, M.D.
Associate Director, EDBP, NIA

OTHERS:

Joan Cornoni-Huntley, Ph.D.
Senior Research Epidemiologist, EDBP, NIA

COOPERATING UNITS (if any)

Department of Epidemiology, Yale University

LAB/BRANCH
PCRA

SECTION
EDBP

INSTITUTE AND LOCATION
NIA, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Planning for development of a body of information on the occurrance and frequency of events related to the last days of life. We are developing data concerning how people die; at home or in a hospital and if in a hospital for how long prior to death, in pain or comfortably asleep or at rest, if in pain the amount, duration, and type of medication used, whether death was expected, medical and paramedical contacts and perhaps items such as costs, visits from friends, presence of family, etc. during the last days. Various attempts at finding suitable populations and developing protocols have occurred during the year. In addition, we commissioned and received from Yale University "Circumstances of Death in the Elderly--A Review of the Literature."

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: As stated in the summary of work.

Methods Employed: Not completely evolved.

Major Findings: These data are not abailable from previous studies.

Significance to Biomedical Research and the Program of the Institute:
To document and describe these critical events. To prepare doctors and patients for the likely sequence thus reducing anxiety and potential errors and waste. To establish the needs of such additional services as hospices, etc.

Proposed Course: Hopefully, in collaboration with the NHLBI, a population suitable for study during which a predicted 1,000 deaths would occur in approximately one year will be identified and a contract developed to conduct this investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 01100-01 EDBP

PERIOD COVERED
10-1-78 to 9-30-79

TITLE OF PROJECT (80 characters or less)
Demographic Patterns of Mortality

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clifford H. Patrick, Ph.D.
Research Demographer, EDBP, NIA

OTHERS:
Yuko Palesch, M.S.
Research Statistician, EDBP, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
PDRA

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA,NIH, Bethesda, MD 20205

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patterns of mortality of males and females of each race and age are being analyzed by major causes of death using standard demographic methodology and vital statistics of the U.S. major conditions afflicting the elderly are being identified by analysis of associated conditions present at time of death.

Project Description

Title Mortality Patterns of the Elderly

Objectives:

To examine the survival patterns of blacks, whites, males, and females by age groups and to determine such factors as the age specific and cause specific mortality.

Methodology:

Examine the vital statistics using standard demographic methodology in analyzing cause specific mortality by age and cohort.

Major Findings:

The underlying causes of death understate the significance of selected diseases for the elderly because of the tendency for mutiple diseases to be present in the aged. There are significant variations in impact of major causes when associated causes, e.g. diabetes, are taken into account, especially by age, race, and sex.

Significance to Biomedical Research:

Research will indicate the major conditions afflicting the elderly at time of death and suggest associated diseases which deserve further research.

Significance to NIA Programs:

The results will suggest areas of aging research deserving greater emphasis and new initiatives.

Proposed Course:

Continuing

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 01150-01 EDBP

PERIOD COVERED
10-1-78 to 9-30-79

TITLE OF PROJECT (80 characters or less)

Cohort Survival Patterns

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clifford H. Patrick, Ph.D.
Research Demographer, EDBP, NIA
OTHERS:
Yuko Palesch, M.S.
Research Statistician, EDBP, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
PDRA

SECTION
EDBP

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is designed to determine the demographic, social, and economic characteristics of surviving members of cohorts relative to the deceased members. This information will be used to model and project future aged populations based on cohort characteristics. The study suggests that additional factors must be analyzed in order to determine the quantitative impacts of the environment on the health of the elderly.

Z01 AG 01150-01 EDBP

Title Cohort Survival Patterns

Objectives;

To determine the characteristics of surviving cohorts over time and cross sectionally-to survey survivors of deceased persons to determine the status of the deceased in their last days of life, and determine differences between survivors and nonsurvivors in demographic and socioeconomic factors.

Methodology:

A survey of survivors of persons recently deceased will be carried out by NCHS Mortality Branch. The instrument will be designed by NCHS, NIA, and other participating agencies using standard field methodologies. As a prelude, Census data and vital statistics on cohort characteristics will be examined in a simulation model being developed.

Major Findings:

Differences in socioeconomic factors among cohorts exist and can be modelled. A theoretical model is being developed to use in simulations of population cohort behavior.

Significance to Biomedical Research

Factors in cohort survival will be identified which should influence the direction of biomedical research towards specific diseases in this study.

Significance to NIA Programs

The information gathered on the last days of life of the deceased and their socioeconomic and demographic characteristics will aid in further NIA research.

Proposed Course:

Continuing

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 01190-01 EDBP

PERIOD COVERED
10-1-78 to 9-30-79

TITLE OF PROJECT (80 characters or less)
Environmental Impact on the Elderly

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clifford H. Patrick, Ph.D.
Research Demographer, EDBP, NIA

OTHERS:
Yuko Palesch, M.S.
Research Statistician, EDBP, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
PDRA

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA/NIH, Bethesda, MD 20205

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An analysis of the environmental impact of pollutants generated in electric energy production was undertaken using county level data on type of facility and mortality rates of the elderly. No significant impact was found in the preliminary analysis of data from coal, oil, gas, and nuclear counties.

Project Description

Title Environmental Impact on the Elderly

Objectives:

To determine the impact on the elderly of alternative energy generation capacity in the U.S.

Methodology:

Standard biostatistical methods utilizing county level vital statistics on mortality of those over age 65 and county level energy generating capacity were utilized in a linear regression framework.

Major Findings:

Alternative energy generating capacity by county had no apparent statistical relationship to mortality of the elderly. Numerous confounding factors were not included in this preliminary study which must be taken account of in future studies in order to statistically test the hypotheses of no effect.

Significance to Biomedical Research:

The hypotheses was to test the significance of energy related pollutants on health, many of which are being studied in the laboratory by other NIH Institutes. A positive finding would suggest certain specific pollutants may be injurous to health especially to the elderly and require additional research into mechanisms and prevention.

Significance to NIA Programs:

The study indicates that additional factors must be considered and analyzed in order to appreciate the impact of the environment on the health of the elderly. Many of these other factors such as migration, occupation, diet, and leisure activities are being addressed by grantees.

Publications:

Quantifying the health effects of energy: the U.S. aged as a special population at risk in SIMS Conference on Energy and Health, (A. Whittemore and N. Breslow, eds.), Philadelphia, 1979., pp. 188-205.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 01200-01 EDBP

PERIOD COVERED
10-1-78 to 9-30-79

TITLE OF PROJECT (80 characters or less)
Future Demographic Structure of the Population

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clifford H. Patrick, Ph.D.
Research Demographer, EDBP, NIA
OTHERS:
Yuko Palesch, M.S.
Research Statistician, EDBP, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
PDRA

SECTION
EDBP

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

U.S. Census Bureau population projections for the years 2050 and 2100 are being used to analyze future health and demographic characteristics of the elderly. The distribution of diseases among the future aged suggests major increases in health facilities and manpower will be necessary unless biomedical breakthroughs are made to reduce the magnitude of the age-specific incidence rates of the major diseases of the elderly.

PHS-6040
(Rev. 10-76)

Project Description

Title Future Demographic Structure of the Population

Objectives:

This project examines the growth in the elderly of the next century and the implications which that growth has for health and socioeconomic impacts.

Methodology:

Census projections to the year 2050 and to the year 2100 will be utilized in conjunction with the socioeconomic and health characteristics of the current population to determine analytically the estimated characteristics of the future population, especially the characteristics of the elderly.

Major Findings:

The aged population of the year 1050 is estimated to be 50 million, with 18% elderly. The aged population of the year 2100 is projected to be 19% over 65, of whom two of every three will be women.

Significance to Biomedical Research:

The large proportion of elderly in the future population and their multiple disease states suggests a major impact on biomedical facilities necessitating improved technology and biomedical research advances to reduce the magnitude of the expected problems.

Significance to NIA Programs

The analysis will show the diversity and magnitude of the elderly population into the future and suggest directions for NIA program development.

Proposed Course:

To be completed in FY 1980.

NIA Annual Report
October 1, 1978 through September 30, 1979

Epidemiology, Demography, and Biometry Program

This is a professional services contract.

Contract Title: Evaluation of Studies of Effective Medical Treatment

Contractor: University of Pennsylvania (Bernard Bloom, Ph.D.)

Money Allocated: $ 8,900

Objectives: To determine the methologically sound studies of effective medical treatment of the elderly. To determine which studies can be considered to have valid outcomes based on the methodology used.

Approach: A literature search was made of studies of medical treatment of the elderly. These studies were then examined to determine if appropriate methodology had been utilized. Those with effective methodology were abstracted in the methodology discussed. Those with inappropriate methodology were also identified and their shortcomings discussed.

Major Findings: There are relatively few studies of effective medical treatment of the elderly which use sound methodologies. Conclusions drawn from studies which have used inappropriate methodologies may be misleading although they are widely quoted. An annotated bibliography was provided to NIA as a consequence of this contract.

Significance to Biomedical Research: If studies are widely cited and accepted as being effective or alternatively ineffective when the basis of the results is inappropriate methodology, then biomedical research may tend toward directions which would otherwise not have been suggested. Therefore, this study is important in determining which studies can be considered reliable in terms of their results and which studies need to be replicated in order to determine if their results hold up under appropriate methological analysis.

Significance to NIA Programs: Ths study indicates those areas of research which need replication and which treatments seem to have been firmly established as effective by appropriate quantification.

Proposed Course: The study was completed in FY 1979

Epidemiology, Demography, and Biometry Program

This is a professional services contract.

Contract Title: Spending Patterns of the Elderly

Contractor: Columbia University (Phoebus Dhrymes, Ph.D.)

Money Allocated: $4,500

Objectives: To determine the sources of income and consumption patterns of the elderly, especially for medical care, food, and shelter.

Approach: Standard microeconomic analysis utilizing the latest Consumer Expenditures Survey of the Bureau of Labor Statistics is being carried out. Estimation of the demand equations including elasticity is being utilized. The elderly household is the unit of observation and is being compared with non-elderly households of the same size, i.e., one household member, two household members, etc.

Major Findings: Race *per se*, taking into account household characteristics, is not a significant factor in the consumption allocation pattern of the elderly. The most important distinction appears to be age, especially 75+, in differences in spending patterns of the elderly.

Significance to Biomedical Research: This data indicates the economic status of older Americans and is useful in calculating cost and benefits of biomedical research breakthroughs which would extend the lives of the elderly. These figures can be used in analyses of the changes in the economic impact of increased longevity resulting from biomedical research.

Significance to NIA Programs: The data are extremely useful to the EDBP program, the Program Planning Office, and the Social and Behavioral Sciences Program because they allow hypotheses concerning the social, demographic, and economic status of the elderly to be examined.

Proposed Course: Completed in FY 1979

NIA Annual Reprot
October 1, 1978 through September 30, 1979

Epidemiolgoy, Demography, and Biometry Program

This is an interagency agreement.

Contract Title: Demographic Aspects Of Aging In Older Populations In The U.S.

Contractor: U.S. Bureau of the Census (Population Division/J. Siegal)

Money Allocated: $100,000

Objectives: To utilize census data to identify the socioeconomic and demographic differentials among the aging population of the U.S. over time. To examine population trends as they relate to population projections for the different age groups. To identify data resources available from the Census Bureau for aging research.

Approach: Standard demographic methodology will be utilized to analyze census data from the 1970 Census and recent Current Population Surveys.

Significance to Biomedical Research: The data will allow the impact of selective biomedical research which is age specific to be calculated for the elderly, such as declines in hypertension due to biomedical breakthrough.

Significance to NIA Programs: The census analysis will indicate the magnitude of the elderly population and the growth over the next century in the elderly in all areas of the nation by demographic and socioeconomic groups.

Proposed Course: Continue in FY 1980

Epidemiology, Demography, and Biometry Program

This is an interagency agreement.

Contract Title: Factors in Institutionalized Elderly By State

Contractor: U. S. Bureau of the Census (H. Wallach)

Money Allocated: $40,000

Objectives: To determine the differentials in institutionalization by state and by age, sex, and race. To examine the literature on institutionalization and to examine the census data on institutionalized population for evidence of factors in the likelihood to be institutionalized.

Approach: In addition to a literature review of completed studies, a factor analysis of State level demographic data from the Census will be used to identify factors which distinguish the institutionalized population from the non-institutionalized.

Major Findings: Striking demographic differences in the rates of institutionalization are discernible by State and region of the country.

Significance to Biomedical Research: This analysis will indicate the significance of age differentials in institutionalization and may suggest areas for additional biomedical research to address senility and other factors in the institutionalization of the older ages.

Significance to NIA Programs: The data will indicate the magnitude of the institutionalized patients and the differentials by demographic and socioeconomic factors as well as selected reasons for institutionalization.

Proposed Course: Continue in FY 1980

NIA ANNUAL REPORT

Report of the Gerontology Research Center

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1.	Office of the Scientific Director	GRC/OSD-1
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3.	Laboratory of Cellular and Molecular Biology	GRC/LCMB-87
4.	Laboratory of Molecular Aging	GRC/LMA-130
5.	Laboratory of Neurosciences	GRC/LNS-170
6.	Clinical Physiology Branch	GRC/CPB-204

NIA ANNUAL REPORT
OCTOBER 1, 1978 through September 30, 1979
GERONTOLOGY RESEARCH CENTER

The Gerontology Research Center experienced a rewarding year and broadened the scope of its overall research commitment with the establishment of two new sections, while the Laboratory of Neurosciences, put in place a year ago to investigate age-related changes in the central and peripheral nervous systems, has rapidly developed into a stable and productive organization.

The newly created Stress and Coping Section in the Laboratory of Behavioral Sciences made striking progress under the direction of Dr. Paul Costa. He successfully recruited a team of Staff Fellows and supporting personnel which enabled the Section to begin investigating personality and the aging individual's ability to cope with and adapt to major and minor stresses and life events. In the past such events were not systematically studied in the Baltimore Longitudinal Study of Aging. In less than a year, however, Section investigators have developed questionnaires, interviews, and psychological tests to accurately assess many of the stresses faced by adults, their coping strategies, and the effectiveness of these strategies for individual members of the Baltimore sample.

In the immediate Office of the Scientific Director, a Section on Experimental Morphology was established late this year. The creation of this unit will help maximize Center investigations related to the morphological changes that occur with aging. In addition, the Section will provide supportive services to scientists in other GRC laboratories who wish to correlate physiological and biochemical phenomona with their morphological consequences. A Senior Staff Fellow, Dr. Susie Humphreys, has been recruited to work in this Section. Her research interests center on cell surfaces and their interactions in adhesion and regulation.

The dynamic leadership of Dr. Stanley I. Rapoport and his recruitment of a half dozen outstanding Staff and Visiting Fellows is responsible for the Laboratory of Neurosciences successful initial year. A variety of studies were initiated including those on the blood-brain barrier and central nervous system function, cerebral metabolism, pharmacology of the central nervous system and peripheral nervous system, synapse development, and CNS binding sites for kainic acid. This Laboratory published 13 papers this year with a number of others now in press.

The status of the oral cavity in longitudinal participants has been one of the few things never studied in this population. Recently, a clinical study to assess age related alterations in oral function was initiated by Dr. Bruce Baum in the Laboratory of Molecular Aging. The fundamental information gained should provide a unique data base of great potential for understanding and perhaps ameliorating the adverse impact of a variety of decremental changes (e.g., loss of oral muscle tonus) seen in some older individuals.

Throughout the GRC there is a growing trend toward collaboration between investigators from a variety of disciplines. This collaboration and cooperation extends further to the NIH, and beyond to the local, national, and international community of scientists. Over the past year, as many as three different Center laboratories pooled their expertise to answer specific questions posed in a single research project. Staff worked closely, also, with at least six of the other ten NIH institutes and seven foreign institutions, as well as more than 15 U. S. institutions of higher learning. Of course, the excellent working relationship between GRC scientists and their colleagues at City Hospitals, Johns Hopkins, and the University of Maryland continues to flourish and grow. This cross-fertilization of ideas and cooperation between investigators helps form the scientific basis needed for solid advances in the aging research field.

The exchange of ideas at GRC was further enhanced by the succession of brilliant scientists from laboratories in this country and abroad who shared their theories and accomplishments during the 34 formal Monday seminars held this year in Baltimore. Nearly as many more seminars were held by the individual branches and laboratories.

It would be remiss to end this brief summary without mentioning with pride some individual achievements by Center staff. Dr. Gunther L. Eichhorn, Chief, Laboratory of Cellular and Molecular Biology, was named Maryland Chemist of the Year by the American Chemical Society. He was honored for his pioneering work in inorganic biochemistry and his compilation of the definitive textbook in the field, Inorganic Biochemistry, (1973). Two key GRC staff received the NIH Merit Award for their special skill without which Center scientists would be hard pressed to carry out their respective missions. The Administrative Office's Bertha Voelker was cited for her conscientious and effective management of critical administrative matters at the GRC. Richard E. Hiner of the Technical Development Section was honored for his highly developed skills in instrument design and fabrication in support of Center research programs. Finally, the Information Office's Daniel S. Rogers was named Handicapped Marylander of the Year by the Governor's Committee on Employment of the Handicapped which cited him for his personal and professional accomplishments, the latter including his direction of GRC information activities.

Office of the Director

Section on Comparative Nutrition

The specific activities of aldolase of cytosols isolated from the livers of 30-31 month old rats were statistically significantly lower than those isolated from 6-12 month old animals. This finding was observed in the three strains (Fisher 344, Wistar and Sprague-Dawley) examined. The monospecificity of the antibody recognized the aldolase in liver cytosol of young adult and senescent rats as a single antigen. Immunoprecipitation of the aldolase of cytosols of young and old rats showed that 13% and 22% more antibody was needed to neutralize 50% of the old as compared to the young enzyme. In addition, the specific activities of aldolase purified from cytosols of old rats was 15% lower than that isolated from young rats. (Although the present study provides consistent evidence for the presence of inactive aldolase in the cytosols isolated from old rats in both studies of immunoprecipitation and purification of the enzyme, the age-associated changes may be considered of marginal functional importance.)

The increase in specific activity due to feeding a high fructose-no fat diet was considerably greater in mice than in rats. Similarly, specific activities of aldolase of cytosols of mice fed a diet containing 4% protein and 70% sucrose were 54-100% higher than those of animals fed Purina Laboratory Chow or a synthetic diet containing 24% protein and 50% sucrose. However, the response to this low protein-high carbohydrate diet was markedly lower in rats than in mice. Indeed, in some old rats feeding such a diet resulted in a markedly reduced specific activity. Also, the reduced response in the specific activity of aldolase in rats is accompanied by a reduced protein content of the cytosol. The protein content of the cytosol of mice was not altered by feeding this 4% protein-70% sucrose diet. This finding suggests a difference in the dietary protein requirements of rats and mice. In fact, the growth curves established in this study clearly indicate that the mouse can support growth more readily on low levels of dietary protein than can the rat.

Further studies will be carried out to establish the effect of induction and age on the specific activity of aldolase. Purified aldolase will be examined by gel electrophoresis, isoelectric focusing, and immunoprecipitation. In contrast to the minimal effects of age on aldolase in rodents, reports indicate this enzyme decreases 50-100% in nematodes. Therefore a nematode colony will be established with the idea of using the nematode as a model system to examine the interaction of nutrition, aging, and altered enzymes.

Kidney glomeruli were examined morphometrically in mice fed one of three diets beginning at one month of age: 1. control diet fed ad libitum; 2. control diet fed on Monday, Wednesday and Friday (intermittently fed); or 3. 4% protein diet ad libitum. Glomerular diameter, podocyte diameter, and density of microvilli, rated on a scale of 1 to 10, were measured at 1 month, 10 months and 24 months of age. Glomerular diameter increased with age as did podocyte diameter and microvillus density (microvillus index). Glomerular diameter, podocyte diameter and microvillus index were significantly lower in the low protein group, while only the microvillus index was significantly lower in the intermittent fed group.

Glomerular diameter and microprojection index (modified from microvillus index since projections in rat kidney varied in diameter) increased with age, confirming data in the mouse. Hypophysectomy reduced glomerular diameter and microprojection index, similar to the reduction following dietary restriction. Hypophysectomy also reduced the frequency of glomerular cells containing age pigment (lipofuscin). Podocytes containing secretory (granular) material did not change in frequency with age but were reduced following hypophysectomy. Basal laminar thickness increased with age but was unchanged following hypophysectomy.

TEM and SEM studies on the kidney will continue. Cyclic AMP in the glomeruli of young, old and hypophysectomized rats or mice will be analyzed. Preliminary evidence suggests brain may be affected differently than kidney by hypophysectomy and, therefore, several brain regions will be studied for evidence of the effect of hypophysectomy on cell structure as well as blood brain barrier.

Studies have been initiated to evaluate the effect of age on the absorption of vitamins A and D. Further examination of changes in vitamin D metabolism with age will measure conversion of 25-OH-D_3 to 1,25 $(OH)_2$-D_3 in young, adult, and senescent female Wistar rats. Changes in either absorption or conversion of vitamin D may be important with respect to the very prevalent problem of osteoporosis in the aged population. Continuation of the present study will examine changes in absorption of water soluble vitamins with age.

Technical Development Section

The Technical Development Section continues to provide maintenance and development services to Center programs. Several new instruments also have been developed this year.

Every program received support from the Section's Mechanical Shop. Particularly significant was the design and fabrication of ergometry apparatus for the Psychophysiology Section, a leg plethysmograph for the Human Performance Section, a muscle chamber for laser diffraction studies in the Cardiovascular Section, and receptor assaying apparatus for the Endocrine Section.

The Center's second computer system became operational. With the Section's assistance, several investigators began using the new system offering increased disc space, memory size, tape speed, and computational capabilities.

Microprocessor computer systems were implemented to meet the instrumentation needs of staff. This relatively new technique allows moderately complicated instrumentation to be designed and fabricated quickly. The following systems were completed and installed this year: (1) Tapping Test - Human Performance Section; (2) Heart Rate Histogrammer - Psychophysiology Section; (3) EM Tissue Processor - Comparative Nutrition Section; (4) Data Collector for stopped-flow spectrophotometer - Biophysics Section; and, (5) Free-Recall Test - Learning and Problem Solving Section.

Other systems in various phases of development include: (1) A system to deliver a multi-exponential infusion rate - Laboratory of Neurosciences; (2) Digital recording and control of exercise ECG testing - Human Performance Section; and, (3) Camera controller - Clinical Immunology Section.

Animal Resources Facility

The Animal Resources Facility maintained full accreditation with the American Association for Accreditation of Laboratory Animal Care. Monthly inventories show that in the Open Animal Colony, care and housing were provided for 14,600 mice, 1,365 rats, 90 rabbits, 50 dogs, 8 monkeys, and 5 cats. Throughout the year the ARF received, maintained, or issued 27,400 mice, 3,106 rats, 1,611 rabbits, 22 dogs, 15 monkeys, and 11 cats.

The oldest mice in the C57BL/6J Mouse Colony are 32-months-old. The Colony population is over 14,000. A total of 473 mice were issued from this Colony. The Wistar Rat Colony continues to grow, and the current population numbers over 12,000 rats. During the year, 8,850 weanling rats were introduced into this colony. A total of 3,091 rats were issued.

Information Office Activities

The GRC Information Office served many publics as the popularity of the NIA and its intramural arm in Baltimore continued to increase with the media, Congress, educational and medical institutions, and the population-at-large.

Nearly 500 people took part in 56 briefings and tours. Visitors ranged from physician members of the American Geriatrics Society to the Congressional Wives Task Force and staff from Congressional offices, to students from as far away as Rider College in New Jersey and high school students from every corner of Maryland. The Information Office presented aging programs for Baltimore County Senior Aides and at various area senior centers.

Stories on the NIA Center aired over WPVI-TV (Philadelphia), WXYZ Radio (Detroit), Prime Time Radio, WBFF-TV (Baltimore), and WAMU-FM (Washington). Contacts for future programs came from WNET-TV (New York), CBS-TV, "Over Easy" KQED-TV (San Francisco), CTV-TV Reports (Toronto, Canada). Also, several Canadian radio stations interviewed GRC staff. Assistance was given to WHA-TV (Wisconsin), and WCVB-TV (Boston).

Print articles appeared in the New York Times, New York Daily News, Chicago Tribune, Baltimore Evening Sun, Baltimore Sunday Sun, Oakland (Calif.) Press, and Family Circle, to name a few. Specialty magazine, journal, or newsletter interest came from Smithsonian Magazine, Modern, Maturity, Modern Medicine, Family Health, JAMA Medical News, Psychology Today, Science News, International Medical Tribune, Nutrition Today, Hospital Practice, Older Americans Report, and Baltimore Magazine.

GRC information personnel indexed 10,000 titles for publication in the bi-monthly Journal of Gerontology section, "Current Publications in Gerontology and Geriatrics," nearly doubling the number indexed last year. Also, Information staff activities included considerable time spent arranging for photos and videotaping at the Center for Institute publications and orientation programs; publication of the internal mimeographed communication, GERON-NEWS; involvement with the GRC Library Committee, NIA EEO Committee, and NIH Women's Advisory Committee.

Library Services

This year the GRC Library processed more than 2,500 interlibrary loan requests submitted by or to universities, colleges, the NIH library, National Library of Medicine, Library of Congress, and other area government libraries. Accounts were established with the Welch Medical and Milton S. Eisenhower Libraries to accommodate staff's requests to reproduce articles.

Installation of a computer terminal and contracts with the National Library of Medicine and Bibliographic Research Services provide online and offline literature retrieval. With MEDLARS' and BRS' numerous data bases, the library now can quickly retrieve literature on aging. Installation of microfilm and microfiche reader/printers provide users with a valuable resource to read and reproduce hard copies of materials stored only on microfilm, including dissertations, government publications, documents, and materials on aging from the Administration on Aging. The library plans to add microforms. Active classification of the library's collection continues and should be completed by the end of the summer.

Outside library visitors totaled 250 for the year, and included officials from Federal, state and local governments, scientists from foreign countries, students, and the general public.

Personnel Activities

During the year, GRC established one new Laboratory and two Sections, reorganized another Laboratory, i.e., Laboratory of Neurosciences, Stress and Coping Section, Experimental Morphology Section, and Laboratory of Cellular and Molecular Biology. Personnel's efforts to recruit for these positions were successful. They are now 95% staffed and operational.

OPM conducted an evaluation of GRC's implementation of DHEW's Three Year Plan in October 1978. Personnel worked closely with OPM to assure GRC employees proper classification.

Personnel counseled employees in the area of formal and informal training opportunities. This counseling provided employees with eligibility requirements, job relatedness, and career objectives. Over 1,500 personnel actions, i.e., promotions, reassignments, new employees, within grade increases, quality step increases, and renewals of temporary employees were processed this year. The Personnel Office serviced some 340 employees and Special Program Researchers.

Personnel also worked with the Equal Employment Opportunity Committee to update the NIA Affirmative Action Plan and to assess programs in effect under the current plan.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AG-00101-03-SCN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Relation between Nutritional State and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. H. Barrows	Chief	SCN, OSD, NIA
	G. C. Kokkonen	Chemist	SCN, OSD, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH
Gerontology Research Center, Office of the Scientific Director

SECTION
Section on Comparative Nutrition

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The specific activities of aldolase of cytosols from the livers of 30-31 month old rats were statistically significantly lower than those isolated from 6 or 12 month old animals. Although these data provide consistent evidence for the presence of inactive aldolase in the cytosols isolated from old rats in both studies of immunoprecipitation and purification of the enzyme, the age-associated changes may be considered of marginal importance. For example, in the former studies between 13 and 22% inactive enzymatic molecules were observed. In the latter studies estimates of their presence were similar; namely; 15%. A greater increase in aldolase activity occurs in mice as compared to rats fed either a high fructose-no fat diet or a low protein-high carbohydrate diet. This latter finding suggests that there is a difference in the dietary protein requirements of rats and mice. Indeed, the growth curves established in this study clearly indicated that the mouse can support growth more readily on low levels of dietary protein than the rat.

PHS-6040
(Rev. 10-76)

Objectives: Attempts were made to establish whether aging is accompanied by the formation of selective errors in the genetic code which may occur with age and/or use.

Methods Employed: The activities of aldolase and the protein contents of the cytosols isolated from the livers of rats (Fisher 344, Sprague-Dawley and Wistar) and mice (C57BL/6 and CBAT$_6$T6) were determined at various ages in animals fed either Purina Laboratory Chow, a diet which contained 24% protein, 4% protein, or a fat free-high fructose-24% casein diet. In addition, aldolase from the livers of the animals was purified by ion-exchange chromatography on a phosphocellulose column using a linear salt gradient followed by substrate elution. Immunoprecipitation of aldolase of the cytosols of the animals was accomplished by the addition of monospecific anti-rat-liver aldolase in varying quantities to a constant amount of enzyme.

Growth rates were established for female 344 Fisher rats and female B$_6$D$_2$F$_1$/J mice fed diets containing either 24% protein, 12% protein, 8% protein, or 4% protein *ad libitum*.

Major Findings: The specific activities of aldolase of cytosols isolated from the livers of 30-31 month old rats were statistically significantly lower than those isolated from 6 to 12 month old animals. This finding was observed in the three strains examined. The monospecificity of the antibody for rat-liver aldolase was demonstrated by double diffusion in gel. This antibody recognized the aldolase in liver cytosol of young adult and senescent rats as a single antigen. The immunoprecipitation of the aldolase of cytosols of young and old rats showed in two studies that 13% and 22% more antibody was needed to neutralize 50% of the old as compared to the young enzyme. In addition the specific activities of aldolase purified from cytosols of old rats was 15% lower than that isolated from young rats. The increase in the specific activity due to feeding a high fructose-no fat diet was considerably greater in mice than rats. Similarly the specific activities of aldolase of cytosols of mice fed a diet containing 4% protein and 70% sucrose were 54-100% higher than those of animals fed Purina Laboratory Chow or a synthetic diet containing 24% protein and 50% sucrose. On the other hand the response to this low protein-high carbohydrate diet was markedly lower in rats as compared to mice. Indeed in some old rats feeding such a diet resulted in a markedly reduced specific activity. In addition the reduced response in the specific activity of aldolase of rats is accompanied by a reduced protein content of the cytosol. The protein content of the cytosol of mice was not altered by feeding this low protein-high carbohydrate diet. This latter finding suggests that there is a difference in the dietary protein requirements of rats and mice. Indeed, the growth curves established in this study clearly indicate that the mouse can support growth more readily on low levels of dietary protein than the rat.

Studies have been initiated but are thus far incomplete to determine the effect of induction and age on the specific activity of purified aldolase of mice. These data indicate that the age decrement (16%) in the specific activity of purified aldolase of mice is a less than reported in the literature (50%).

In addition the following two major preliminary observations have been made: 1) in young animals there is a markedly higher specific activity of purified induced enzyme (1.90) as compared to that of purified normal enzyme (1.46); 2) in old animals this increment is not as evident in the purified induced enzyme (1.39) as compared to that of purified normal enzyme (1.22).

Significance to Biomedical Research and the Program of the Institute: The error catastrophe theory of aging proposed by Orgel has led to investigations of the association between age and altered enzymes. Among the enzymes which may be altered with age is aldolase. Age related decreases in the specific activity of aldolase have been reported in nematodes and the livers of mice and rabbits. However such decreases have been reported not to occur in the livers of rats. If the observed age related decreases in the specific activity of aldolase represent a fundamental biological mechanism it must occur in the rat. The results obtained during the past year clearly show that the specific activity of this enzyme in the rat is decreased with age. The decrease is the result of structural changes of the enzyme with age; however the mechanism responsible for this observation remains unknown. During the past 15 years a great number of experiments have been carried out to determine the degree to which aging is accompanied by errors in the transcription of genetic information. In spite of marked inconsistencies among species, enzymes, and methods used to assess the presence of altered enzymes, errors in the transcription of genetic information necessary for the synthesis of tissue proteins still remains a possibility.

Proposed Course: The preliminary studies carried out to establish the effect of induction and age on the specific activity of purified aldolase will be completed. The data suggest that there are structural changes in the molecule that occur both with age and induction as well as their interrelationship. As a first attempt to determine the characteristics of these apparent various forms of aldolase, purified aldolase will be characterized by gel electrophoresis, isoelectric focusing, and immunoprecipitation. In addition attempts will be made to activate the purified aldolase isolated from old animals to that observed in young induced mice.

Publications:

Barrows, C. H. and Kokkonen, G. C.: Diet and Life Extension in Animal Model Systems. Age 1: 131-143, 1978.

Barrows, C. H. and Kokkonen, G. C.: Effect of Age on the DNA, RNA, and Protein Content of Tissues. In Florini, J. (Ed.): Handbook of the Biology of Aging, West Palm Beach, CRC Press, in press.

Barrows, C. H. and Kokkonen, G. C.: Genetic Information and Life Span. In Segal, M. (Ed.): Reviews on Research in Pharmaceutical Science, in press.

Barrows, C. H. and Kokkonen, G. C.: Studies of Aldolase in the Livers of Rats and Mice. Age in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AG-00141-02-SCN

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Effect of Aging on Vitamin Absorption

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. B. Fleming	Staff Fellow (EOD 5.8.79)	SCN, OSD, NIA
Other:	C. H. Barrows	Chief	SCN, OSD, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Office of the Scientific Director

SECTION

Section on Comparative Nutrition

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland, 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The absorption of vitamins A and D will be studied in young (6 month), adult (12 month), and senescent (24 month) female Wistar rats. The effect of aging on both rate and amount of absorption of physiological doses of these vitamins will be determined.

PHS-6040
(Rev. 10-76)

Objectives: The objective of this project is to determine the effect of aging on absorption of vitamins A and D.

Methods Employed: Rats will be dosed via stomach tube with radioactive forms of vitamins A and D. At predetermined times following dosing rats will be sacrificed and the gastrointestinal tract, blood, liver, kidney, and feces will be analyzed for content of the administered vitamins.

Major Findings: Preliminary studies have been completed. Major absorption studies are in progress.

Significance to Biomedical Research and The Program of the Institute: Research on the effect of aging on gastrointestinal absorption is limited and inconclusive. An effect of aging on absorption may explain observed reduced levels of various vitamins in the plasma of aged individuals and could result in altered recommendations for nutrient intake with potential for improved health in this group. Knowledge of the effect of aging on gastrointestinal absorption could also have important implications with respect to drug absorption.

Specifically, altered absorption of vitamin D with age could have important implications with regard to the problem of osteoporosis in the aged population.

Proposed Course: Several other studies are in the planning stages. A continuation of the present study will involve examination of changes in absorption of the water soluble vitamins with age.

Further examination of changes in vitamin D metabolism with age will consist of measurement of the conversion of 25-OH-D_3 to 1,25 $(OH)_2$-D_3 in young, adult, and senescent female Wistar rats. Changes in either absorption or conversion of vitamin D may be important with respect to the very prevalent occurrence of osteoporosis in the age.

Additional studies designed to examine the relationship of nutrition and aging include an investigation of the corticosteroid response to immobilization of protein-restricted female rats. Although protein restriction is known to result in increased lifespan, the functional state of various systems throughout the lifespan of these animals has not been fully explained. A nematode colony will be established and the nematode will be used as a model system in which to examine the interaction of nutrition and aging.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00142-02-SCN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Fine Structural Changes in Aging Cells and Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Johnson, Jr.	Senior Staff Fellow	SCN, OSD, NIA
Other:	C. H. Barrows	Acting Chief, SCN	OSD, NIA
	S. I. Rapoport	Chief LNS	LNS, NIA
	R. G. Cutler	Research Chemist	SCN, OSD, NIA

COOPERATING UNITS (if any)
J. Miquel, NASA Ames Research Center, Moffett Field, California
D. Brandes, Dept of Pathology, Johns Hopkins University, Baltimore, Maryland

LAB/BRANCH
Gerontology Research Center, Office of the Scientific Director

SECTION
Section on Comparative Nutrition

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Md. 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.00	1.00	2.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range goal of this project is to examine, by electron microscopy, cells from tissues of mice, rats, monkeys and humans of various ages, to determine what similarities and differences exist, in terms of age related, structural changes, between different cell types of individual species and between similar cell types of the different species. Comparisons are being made between in-vivo models and in-vitro models at both the transmission and scanning electron microscopic levels. The effects of diet, drugs and and surgical intervention on the rate of aging at the cellular structural level are being examined.

PHS-6040
(Rev. 10-76)

Objectives: This research is oriented towards the study of cellular and tissue aging at the electron microscopic level. Anatomy is one of the most basic of the biomedical sciences and the electron microscope is our most powerful tool for study in this discipline. Very little is known about fine structural (electron microscopic) changes which occur with aging. This is true both at the transmission electron microscopic (TEM) level and at the scanning electron microscopic (SEM) level. Whole systems in the body are still almost unstudied with these techniques. Therefore, we wish to begin filling in the gaps in these unstudied systems with general baseline data and at the same time, search out the answers to questions involving specific intracellular activities such as the relationship of lysosomes, lipofuscin and mitochondria. We also wish to vary the environment, which, broadly, includes diet, drug treatment, and surgical intervention to determine if the rate of cellular structural aging can be manipulated.

Methods Employed: Mice and rats of various ages ranging from 1 month to 40 months have been sacrificed by aldehyde perfusion. At present, these include untreated controls, mice which have been on dietary restriction (intermittently fed or low protein diet, *ad-lib*) and rats which have been hypophysectomized (pituitary removed). Since diet is one of the most powerful factors influencing life span, the dietary manipulation experiments are of great importance in this program. Hypophysectomy has recently been shown to return biochemical and physiological variables in old rats to levels of their younger counterparts. Therefore, we are presently comparing histological effects of dietary restriction and hypophysectomy in aging animals. For the hypophysectomy study, female rats were hypophysectomized at 19 mo and sacrificed at 23 mo of age. Between surgery and sacrifice the animals were given, in their drinking water: hydrocortisone, deoxycorticosterone acetate, L-thyroxine, sodium chloride, sodium phosphate, sodium citrate, potassium chloride, magnesium acetate, and calcium chloride. Three weeks before sacrifice, the hormones were discontinued in the drinking water (salts were continued) and the hormones were given on a per kilogram body weight basis daily by injection. 23 mo old and 9 mo old intact controls were also sacrificed.

Major Findings: we examined (by SEM) the kidney glomeruli of mice which had been on one of three diets since 1 mo of age: 1. control diet fed *ad libitum*; 2. control diet fed on Monday, Wednesday and Friday (intermittent fed); or 3. 4% protein diet. Glomerular diameter, podocyte diameter and amount of microvilli, rated on a scale of 1 to 10, were measured at 1 mo, 10 mo and 24 mo of age. Glomerular diameter increased with age as did podocyte diameter and microvilli (microvillus index). Glomerular diameter, podocyte diameter and microvillus index were significantly lowered in the low protein group, while only the microvillus index was significantly lowered in the intermittent fed group. These data are shown in the following table:

The Effect of Various Dietary Regimens on Morphological Variables Examined by Scanning Electron Microscopy

Age	Diet	Glomerular diameter (μ)	Podocyte diameter (μ)	Microvillus index
1 mo	Controls	56.5 ± 0.55[a]	11.63 ± 0.50	2.96 ± 0.52
10 mo	Controls	61.3 ± 1.88	13.12 ± 0.96	5.29 ± 0.32
	Low protein	52.6 ± 3.47	11.63 ± 0.63	4.48 ± 0.96
	Inter. fed	61.4 ± 2.98	12.38 ± 0.41	3.13 ± 0.07
24 mo	Controls	78.3 ± 5.24	14.06 ± 1.13	6.46 ± 0.27
	Low protein	57.7 ± 2.54	11.06 ± 0.08	4.56 ± 0.06
	Inter. fed	67.2 ± 4.09	12.55 ± 1.39	2.53 ± 0.22

[a] Mean ± Standard error of the mean

Age related changes are statistically significant for glomerular diameter ($p<.01$) and microvillus index ($p<.005$). At 24 mo of age, significantly lower glomerular diameter ($p<.05$) and podocyte diameter ($p<.05$) were found in low protein fed animals compared with controls. The microvillus index was significantly lower in both restricted groups compared to controls. This occurred at 10 mo ($p<.025$) and 24 mo ($p<.005$) for intermittent fed animals, and at 24 mo ($p<.005$) for low protein fed animals.

In the hypophysectomy study, glomerular diameter and microprojection index (modified from microvillus index since projections in rat kidney varied in diameter) increased with age, confirming data in the mouse. Hypophysectomy reduced the glomerular diameter and microprojection index, similar to the reduction following dietary restriction. Hypophysectomy also reduced the frequency of glomerular cells containing age pigment (lipofuscin). Podocytes containing secretory (granular) material did not change in frequency with age but were reduced following hypophysectomy. Basal laminar thickness increased with age but was unchanged following hypophysectomy. These data illustrated in the following table:

Measurements in Young, Old and Hypophysectomized Rats

	Glomerular Diameter	MPI	Basal Laminar Thickness	Podocytes with Dense Bodies
Young (N=3)	118.3μ ± 5.08*	1.03 ± 0.03	0.27μ ± 0.03	7.5% ± 2.25
Old (N=3)	150.9μ ± 3.46	2.27 ± 0.99	0.48μ ± 0.03	35.0% ± 8.38
Hypop. old (N=3)	103.8μ ± 5.87	1.05 ± 0.05	0.46μ ± 0.02	15.4% ± 4.19

Table 2 continued:

	Podocytes with Granular Material	Mesangial Cells with Dense Bodies	Endothelial Cells with Dense Bodies
Young (N=3)	14.9% ± 6.40	27.2% ± 7.53	6.0% ± 0.86
Old (N=3)	14.7% ± 2.13	43.0% ± 9.58	17.2% ± 3.49
Hypop. old (n=3)	4.1% ± 2.04	22.9% ± 4.92	3.8% ± 0.0

Statistical significance (Number indicates column, Y=young, O=old, H=hypophysectomized). The comparison listed below are significant at the .05 level by the Mann-Whitney U test.

1-Y-O
1-O-H
2-Y-O
2-O-H

3-Y-O

4-Y-O
5-Y-H
5-O-H
7-Y-O
7-Y-H
7-O-H

* ± Standard error of the mean.

Significance to Biomedical Research and the Program of the Institute: The results of these studies indicate that in dietarily restricted animals, kidney glomerular structure of older subjects appear more like their younger counterparts, by scanning electron microscopy. Changes in the amount of microvilli have functional significance since it has been shown that higher numbers of surface microvilli on kidney cells grown in culture correlate inversely with intracellular cyclic AMP. In our own studies, in vitro, we have found that human lung fibroblasts (IMR-90) have larger numbers of microvilli on their surface as the population doubling level increases. Thus, including the hypophysectomy data, the microvillus index as a function of age has been confirmed in three systems; mouse kidney, rat kidney, and fibroblasts in vitro.

The hypophysectomy study represents the first report (submitted) which shows a reduction of age pigment in old animals without any drug treatment (centrophenoxine). This refutes the idea that lipofuscin is a totally undigestible residue that is present for the life of the cell. Rather, it would appear that some mechanism can be mobilized in the cell to solubilize and digest, or to excrete these residues.

Proposed Course: The TEM and SEM studies on the kidney shall be continued. Cyclic AMP in the glomeruli of young, old and hypophysectomized rats or mice will be analyzed. Preliminary evidence suggests that the brain may be affected differently than the kidney by hypophysectomy and therefore several brain regions will be studied for evidence of effect of hypophysectomy on cell structure as well as blood brain barrier.

Publications:

Johnson, J.E., Jr.: Transmission and scanning electron microscope preparations of the same cell culture. Stain Technology, 1978, 53, 273-278.

Johnson, J.E., Jr.: Fine structure of IMR-90 cells in culture as examined by scanning and transmission electron microscopy. Mechanisms of Ageing and Development, 1979, 10, 405-443.

Laboratory of Behavioral Sciences

This Laboratory continues to maintain an active and diverse program. The Section on Stress and Coping which was created last year now includes a staff fellow and a visiting foreign fellow. Collaborative activities are a major component of the Laboratory programs. Among collaborating units are: 1) The Cardiology, Dermatology, Gastro-intestinal, Psychiatry and Urology Divisions of Baltimore City Hospitals; 2) The Department of Medicine of Columbia Medical Plan; 3) The John F. Kennedy Institute of Johns Hopkins University School of Medicine; 4) The Human Development Program of the University of Maryland; and 5) The Department of Psychology of the University of Baltimore. One reflection of this collaboration has been an influx of guest workers, Intergovernmental Personnel Act Fellows and Visiting Foreign Fellows. The involvement of so many scientists from such diverse sources is contributing importantly to the prominence of gerontological research within the community of behavioral scientists. For example, this Laboratory is one of the leading research centers in the world in the field of Behavioral Medicine: the application of behavioral principles and methods to the care and treatment of medical patients.

In keeping with its role in the field of Behavioral Medicine, this Laboratory is carrying out a wide-ranging program of clinical research. This research includes studies of personality characteristics in relation to various, clinically relevant aspects of adult human behavior, and it also includes studies designed to evaluate behavioral treatment interventions among diverse clinical populations. One project has begun to define the personality correlates of memory decline in the longitudinal study of men drawn from the Baltimore Longitudinal Study of Aging (BLSA). Among 52 men who were over 70 yrs at first study, it was found that those who had personality traits indicative of higher general activity, greater restraint and greater masculinity were less likely to show a decline in visual memory performance over a six year period. Two studies have explored the question of personality stability and age using BLSA participants. This is an important question because the belief that personality changes with age is widely held among behavioral and social scientists. This view often has led to conclusions that changes in personality traits in older subjects are the result of "normal aging" rather than that these changes are possible pathognomonic signs. Two longitudinal studies over the age range of 17 to 85 years, and based on six year and 12 year test-retest intervals have shown that on a variety of personality trait measures there is considerable evidence of personality stability at all ages: correlation coefficients ranged from 0.6 to 0.9. It is now well established that self-perceptions of health are major factors in health maintenance and in treatment compliance. Since older subjects are more likely to be ill, one important question concerns the relationship between self-perception of health and age. Six and 12 year longitudinal studies of BLSA participants ranging from 17 years to 97 years have shown that there is a correlation between age and higher levels of perceived health problems and illness complaints: These complaints tend to be organ-specific and tend to be primarily sensory, cardiovascular or genito-urinary. This is in sharp contrast to the complaints of neurotic subjects which tend to be more generalized and which also emphasize fatigue as a major symptom. Thus, the notion that older subjects "merely complain a lot" and are "just neurotic" is not supported by these findings.

In addition to the preceeding studies which looked at clinically relevant issues in a general population, three studies were carried out with specific patient populations. Several years ago this Laboratory developed a behavioral treatment for fecal incontinence in medically impaired patients which has now been shown to be the most effective treatment available for such patients. One study carried out by a guest worker in the past year has shown that with appropriate modification this technique can be applied in patients with meningomyelocele, a congenital defect of the spinal cord which often results in fecal incontinence from birth. Another aspect of this research is that experience with these neurologically damaged children should be useful in studies of older adults with lesions of the central nervous system. A second study has been completed with patients diagnosed as having irritable bowel syndrome (IBS). This disease is one of the most common in gastro-enterological practice, and it is very difficult to characterize or to treat. A project completed this year will help to improve diagnosis, and it may eventually lead to the development of a rational therapy. Colonic motility studies have shown that IBS patients are more likely to have spontaneous contractions of the colon than do normals. This year, an LBS guest worker has shown that patients with diarrhea have different motility patterns than do patients with constipation in response to distension of the colon. It also was shown that there was a relationship between amount of hypermotility and severity of clinical symptoms. Also interesting was the finding that while IBS patients showed more evidence of psychophathology than did age and sex matched normals, there were no differences in psychopathology between patients with constipation and patients with diarrhea. A large field study evaluating the behavioral interventions of biofeedback and relaxation in the treatment of high blood pressure is nearing completion. Although there are no findings from the treatment phase of the study, there are results now available from the baseline phase. These results have shown that one can obtain highly reliable measurements of blood pressure from patients who have been taught to monitor their own pressures. Of particular interest is the consistent finding that there are positive correlations between age and systolic blood pressure and age and systolic blood pressure lability. However, no such correlations are seen between age and diastolic pressure. Another finding of interest is that there are frequently wide discrepancies between self-determined blood pressures and professionally determined pressures: Self-determined pressures are significantly lower than professional pressures, but there are wide individual differences. Finally, our results have shown that there is a significant interaction between time of day and level of blood pressure. Most patients have their highest pressures in the middle of the day which is contrary to the widely-held, clinical belief that pressures rise throughout the day and are highest in the evening.

It is very clear that individual behavior is a major determinant of morbidity and mortality. Factors such as medical-treatment compliance, self-restraint and level of usual activity all influence health. It is therefore not surprising that there is considerable interest among LBS scientists in the interaction between behavior and morbidity or mortality. One program has focussed on the interrelationship betwen diet and exercise on rodent survival; a second program has studied the question of whether monkeys or men can be taught to attenuate the heart rate response which normally occurs during exercise. One project explored the interactions between diet control and exercise on survival in rats. It was seen that rats which were subjected to periodic fasting lived 50% longer than did ad libitum controls. Exercise did not affect survival in the fasted animals; however, fasted animals were significantly more vigorous late in life than were non-fasted controls. Interestingly, exercise did increase survival among ad libitum-fed, control animals although the beneficial effects of exercise were not comparable to the effects of periodic fasting. The finding that periodic fasting increases survival also has been shown to be true for various inbred and hybrid strains of mice including one strain which becomes obese at maturity. Just as in the case of the rats, periodic fasting led to a 50% increase in life span. There is a major thrust in this country and elsewhere to improve cardiovascular performance through exercise. Although it is not clear that exercise increases survival in man, it is likely that exercise does enhance subjective well-being. Much of the improvement in exercise-related cardiovascular performance is neurally-mediated although there also is unquestionable evidence that exercise also improves mechanical performance of working muscles. However, it is not at all clear how much of the improvement in neurally-mediated cardiovascular response is attributable to physical conditioning and how much is ascribable to behavioral conditioning. Two completed studies in normal man and one, ongoing project with monkeys have shown that behavioral conditioning comprises a major aspect of the neurally-mediated exercise effect. Physically untrained subjects who were taught to attenutate their heart rate responses while exercising were able to exercise at workloads equal to the loads for control subjects while keeping their heart rates 20% below the rates of the controls. In a second study with highly trained athletes the finding that behavioral conditioning led to lower heart rates was replicated. Furthermore, in this second study it was found that following behavioral conditioning, the athletes were able to transfer their skills to a graded exercise stress test; behaviorally conditioned subjects had lower heart rates throughout the stress tests than did the control subjects. Some additional findings of importance were: 1) In both studies, when the control subjects were behaviorally trained to lower heart rates, they performed comparably to the experimental subjects; 2) In both studies, the product of heart rate and systolic blood pressure (an index of heart work) was lower in behaviorally trained subjects than it was in control subjects; 3) In the second study, oxygen consumption was lower during the post-behavioral training stress tests than it was prior to training indicating a significant increase in efficiency of exercise performance. In a second project in this program, monkeys were trained to exercise and then trained to lower heart rate. Although this project is not completed, preliminary results with two animals indicate that they can attenuate their heart rates while exercising. In another, ongoing project patients with angina pectoris and myocardial heart disease also are being trained in order to see if these patients, who have severely limited exercise capacities, also can improve their exercise performances.

Two, rather diverse projects are exploring age-related, behavioral adjustments on two psychologically significant variables, motivation and problem solving. A major assumption in animal research in gerontology is that motivation declines with age. In a study of age differences in activity it was shown that old rats will work the same amount of time to obtain food rewards as will young rats. However, the older animals work differently than do the younger animals: the older animals work for shorter periods--i.e., they take longer work breaks--but by the end of the 24 hour test periods, they have attained the same total amount of rewards. Thus, while physical ability is reduced in the older animals, motivational level is not different in the two age groups. It is well-known that older people show reduced abilities to solve complex, conceptual problems. A systematic analysis of this performance has shown that elderly subjects could successfully solve such problems. In order to accomplish such tasks the older subjects will use strategies which trade-off rate of problem solving and error rate--i.e., in order to reduce error rate, these subjects work more slowly. Young people do not need to make this trade-off, and very old people (in their 80s or 90s) seem not to be able to make the trade-off. These findings suggest that many old peole can be taught how to solve difficult problems by encouraging them to increase work time to reduce error rate.

There are unequivocal changes in physiological response systems with advancing age. One system which has been shown in this Laboratory to be age-related is the dopamine, neurotransmitter system of the brain. This system is important in controlling motor activity. A second system in which change is age-related is the sudomotor system: There are age-related declines in the ability of old subjects to sweat in response to eliciting stimuli. Two projects are exploring the physiological mechanisms underlying these age-differences. In one project experiments have indicated that the age-related decline in dopamine, neuro-transmitter activity in the rat may be associated with the release or metabolism of dopamine at synaptic junctions as well as with a decline of dopamine receptors in the brain. The analysis of the age-related decline in sudomotor activity in man is technically difficult to resolve because it has not been possible to measure directly sweat gland activity in man. In the past year an LBS investigator has developed an experimental model for measuring this response in the intact person. Experiments are now being planned to characterize sweat gland function in various age groups.

Several projects have been carried out in the monkey to identify the neural mechanisms which mediate learned control of heart rate. One project which was recently completed has separated the role of somato-motor activity from attentional processes. Experimental findings suggest that increases in motor activity mask attentional factors in heart rate control during conditional heart rate speeding; but when the monkey is trained to slow its heart rate, it can be seen that attentional factors greatly influence the ability of the animal to slow. Data from another, on-going project suggest that animals can learn to slow their heart rates even while being electrically stimulated in certain areas of the brain which normally induce heart rate increases. Since this effect is true for some brain areas but not all areas, it should be possible to use the behavioral strategy of heart rate conditioning to trace the pathways of central neural control of the heart. This has been a very productive year for the scientific programs of this Laboratory. Many of the projects cited above are yielding exciting findings which should enable us to improve the well-being of aging people. If projected plans to establish a clinical research facility to test various behavioral treatment procedures in elderly subjects comes to fruition, we can expect to make major advances in future years to mollify the infirmities of late life which prevent so many older persons from enjoying their last years.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00061-17 LBS

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Behavioral Genetics and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles L. Goodrick	Research Psychologist	LBS GRC NIA
OTHERS:	None		

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Learning and Problem Solving

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.10	.50	.60

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major purpose of this project is to determine behavioral differences throughout the lifespan for populations of mice which differ in genetic structure, and which also differ in longevity. The present topics of interest concern the relations of growth rate, maximal body weight and longevity for mice fed low levels of dietary protein and for groups fed adequate dietary protein, in addition to studies of aging and voluntary wheel exercise, and ethanol preference.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The principal objectives of this project are: (1) to determine group differences for behavioral traits and longevity among inbred strains of mice; (2) to determine heritability (degree of genetic determination vs. degree of environmental determination), mode of inheritance (e.g., overdominant, dominant, intermediate, or recessive), and number of segregating units (gene blocks) controlling a particular trait (e.g., longevity); and (3) to examine relative behavioral differences among mouse strains as aging progresses. Other objectives include determining the influence of diet (e.g., protein available) on behavioral traits, growth, and longevity; and identifying single-gene influences upon behavioral traits, growth, and longevity.

Methods Employed: Inbred mice (C57BL/6J and A/J) of a high degree of homozygosity are maintained under uniform environmental conditions. The animals are tested behaviorally during one period of their life span, viz, when mature, mature-old, or aged. Old age is determined as the 50% mortality point for groups maintained throughout their life span. Statistically reliable techniques have been developed to determine behaviors relevant to natural selection such as exploration, general activity level, emotionality, simple or complex problem solving ability and taste preference. The use of segregating F_2 hybrid groups allows an estimate of the mode of inheritance, e.g., dominant or intermediate, and the number of gene blocks or segregating units controlling behavioral traits or life span. For studies in which protein intake is varied for groups of inbred and hybrid mice, isocaloric synthetic diets with 4% (low protein), 26% (control), and 48% (high protein) casein are used. Numerous kinds of mutant mice are maintained on the C57BL/6J background at the Jackson Memorial Laboratories, Bar Harbor, Maine. Our work has concentrated on the albino, beige, yellow and obese mutations. In addition, inbred and hybrid mice are used to test the hypothesis that protein malnourishment increases longevity due to slowing of the rate of growth. Studies of behavioral differences as a function of dietary protein and stage of development are also in progress.

Major Findings:
A. Inbred mice (A/J and C57BL/6J) and hybrid mice (F_1, A/J C57BL/6J) were maintained on low, normal, and high protein diets throughout the life span (N=180). Longevity was found to be inversely related to level of protein. The data will be reported in detail next year. There are presently 4 mice still alive, all from the F_1 low protein group.

B. Obese and yellow mutations and littermate controls were started on periodic fasting (fed every other day) at maturity (20 weeks old). The effect of this procedure was to increase longevity of each group (total N = 40). For obese mice, the increment (above the longevity of *ad libitum* fed mice) was 50.4%, and 48% + for control littermates (some of this group are still alive). Yellow mice obtained an increment in longevity of 32.3% over *ad libitum* fed mice with control littermates at 45.6%. This increment was not dependent on body weight decrement because obese mice maintained a high level of body weight until late in the life span. A small number of mice periodically fasted and some mice fed *ad libitum* are still alive, so the final results will also not be ready until next year.

Significance to Bio-Medical Research and Program for the Institute: The study of the genetics of behavior and longevity allows an assessment of: (1) the mode of inheritance (i.e., dominant, intermediate, etc.) for the factor studied; (2) the relative importance of hereditary and environmental factors; and (3) the number of genes or gene blocks which control the factor studied. Lack of adequate dietary protein is a condition which affects a large proportion of the world population. This project attempts to determine the effect of diet (such as different proportions of protein in the total diet) during particular stages of the life span upon behavior and longevity for animal populations which differ in genetic constitution. Studies of single gene mutant animals are important because they allow the assessment of a specific genetic locus for physiological or behavioral factors.

Proposed Course of Project: Other inbred strains and F_1 hybrid groups are being studied to determine the generality of mode of inheritance of behavioral factors. Cross-sectional and longitudinal studies of mouse behavior will continue with various mouse strains. The longevity of inbred and hybrid groups are also being determined. Experiments with low, normal, and high protein diets should determine: (1) the effect of varying protein diets upon behavior at maturity after access to these diets during various stages of development, and (2) the immediate effects upon behavior of a diet of low, normal or high protein for young or aged mice.

Publications:

Wax, T. & Goodrick, C. Nearness to death and wheelrunning behavior in mice. Experimental Gerontology, 1978, 13, 233-236.

Goodrick, C. Effect of voluntary wheel exercise on food intake, water intake, and body weight for C57BL/6J mice and mutations which differ in maximal body weight. Physiology and Behavior, 1978, 21, 345-351.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AG 00062-06 LBS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Daydreaming and Aging: Normative and Experimental

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leonard M. Giambra	Research Psychologist	LBS GRC NIA
Other:	Bernadette Cider		Towson State Univ.
	Eric Klinger	Prof. of Psychology	University of Minnesota
	Beth Stone	Psychologist	Macquarie Univ. Australia

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Learning & Problem Solving

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS: .6	PROFESSIONAL: .5	OTHER: .1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this work is to determine the parameters of spontaneous-thought-intrusions (daydreaming) and related mental activity such as insight, intuition, mindwandering, and curiosity. This is accomplished through the use of retrospective questionnaires and experimental manipulation. Topics of present interest are: (a) racial, religious, ethnic, and socio-economic influences on daydreaming, (b) the relation of sexual daydreaming and sexual activity to the menopause, (c) the relation of daydreaming incidence to tasks of varying vigilance requirements, and (d) the dimensions of ongoing thoughts on a life-span sample.

Project Description:

Objectives: The goals are: (1) to determine the incidence and content of daydreaming in specific subpopulations (e.g., young, middle aged, elderly) from various socio-economic classes, various races, etc; (2) to attempt to relate these differences in daydreaming to any underlying mechanisms such as physiological state, education, cultural values and beliefs, differential daily experiences, and (3) to investigate experimentally variables which normative studies have indicated may be potent determiners of daydreaming.

Methods Employed: The normative aspects of daydreaming are determined through the use of a structured self-report. Each participant completes a 21 item biographical questionnaire and a 344 item Imaginal Processes Inventory (IPI) which has both specific and general items concerning daydreams, night-dreams, fantasies, etc. There are 28 scales in the IPI. Each item has five choices which are points on a continuum implying frequency or quantity. The choices were assigned values of 0, 1, 2, 3, or 4.

In a vigilance task, the subject must detect changes in visually or auditorially presented material. For example, the subject may see a stimulus designated "A" flash on a projection screen at a specific rate; sometimes, however, another stimulus designated "B" will occur instead of A. The subject's tasks are to detect and report when B appears and to report, by means of a button press, the occurrence of all task unrelated thought intrusions. The proportion of B's can be controlled as well as the number of stimuli presented per minute and the interval during which stimuli remain in view. These factors determine the difficulty and tediousness of the vigilance task and are expected to be related to the incidence of daydreaming.

Major Findings:
I. Racial, religious, ethnic, and socio-economic effects on daydreaming. Data from more than 1800 people who responded to the Imaginal Processes Inventory and to a biographical questionnaire were analyzed to determine unconfounded differences due to religious, racial, ethnic, and socio-economic differences. Inferential statistical analyses were carried out to determine reliable differences.

When whites were classified by (a) Population of place of residence, (b) Religious affiliation, (c) Highest educational degree attained, (d) Annual income, (e) Self-described socio-economic status, and (f) Number of years of education, different patterns of daydreaming and related mental activity as measured by the 28 scales of the Imaginal Processes Inventory were found. This was determined by analyses of variance where the independent variables were the demographic ones identified above, sex (male, female), and age (17-23, 24-44, 45-64, and 65-92 years) and the dependent variables were the subjects' scores on the 28 scales of the IPI. A total of 52 statistically significant effects were obtained which involved the demographic variables. Seven IPI scales were not affected by these demographic variables; they were: Positive Reactions in Daydreams, Present Orientation in Daydreams, Achievement Oriented Daydreams, Hallucinatory-Vividness of Daydreams, Sexual Daydreams, Heroic Daydreams, and Self-Revelation tendencies. Because highest degree obtained had the greatest number of significant effects and because it is the most stable of the demographic variables investigated, this report is restricted to the eleven significant effects involving highest degree. Highest degree had a simple (main) effect on Acceptance of Daydreaming, Problem Solving Daydreams, Future in Daydreams, Mindwandering, and Boredom. People with an M.A./M.S. degree showed less Mindwandering and Boredom than people with High School and B.A./B.S. degrees. Those with M.A/M.S. degrees showed more Problem Solving Daydreams, Future in Daydreams and Acceptance of Daydreaming than those with B.A./B.S. degrees or High School education. In all the simple effects mentioned above, no difference was found between B.A./B.S. and High School groups. The significant interaction of sex with highest degree for Scale 11 indicated that females with M.A./M.S. degrees had more Future in Daydreams but not males. The significant interaction of sex, age, and highest degree on Acceptance of Daydreaming showed that among those with a masters degree, males 65-92 years of age and females 24-44 and 45-64 years of age did not have greater values than their counterparts with less formal education. The interaction of sex, age, and highest degree also was significant for Daydreaming Frequency. For females the B.A./B.S. group and the M.A./M.S. group showed equal Daydreaming Frequency means while those females with a High School education were equal for 24-44 year olds, smaller for 45-64 year olds, and greater for 65-92 year olds; males showed no pattern. Age and highest degree interacted for Scale 26 (Distractibility) such that for 24-44 year olds a higher degree resulted in lower Distractibility, for 45-64 year olds the M.A./M.S. degree group had greater Distractibility, while for 65-90 year olds the B.A./B.S. degree group had somewhat higher Distractibility than the High School group and a much higher Distractibility than the M.A./M.S. group.

Hostile Daydreams had a significant sex by highest degree interaction, and Bizarre-Improbable Daydreams had a significant age by highest degree interaction. Hostile Daydreams increased from H.S. to B.A./B.S. for both males and females; however this scale decreased for males in the M.A./M.S. group, whereas females with M.A./M.S. degrees had by far the highest Hostile Daydreaming scores. For 65-92 year olds as education increased Bizarre-Improbable Daydreams declined, for 45-64 year olds Bizarre-Improbable Daydreams increased then decreased with education while for 24-44 year olds the reverse pattern was found.

The analyses described above were restricted to whites since blacks comprised only a relatively small portion of the total sample. Racial, i.e., Black-White, differences were determined by taking black sample members and matching them to White sample members of the same age, sex, and the appropriate category of the six demographic variables considered above. This resulted in a matched sample of Blacks and Whites in two age groups (17-23 and 24-34 years). There were 34 males 17-23 years old, 52 females 17-23 years old, 8 males 24-34 years old, and 18 females 24-34 years old of each race. Analyses of variance (harmonic mean, unequal n) using age, sex, and race as independent variables were carried out with the 28 IPI scales as dependent variables. Thirteen significant main effects of race or interactions between race and either age or sex were obtained on twelve IPI scales. Blacks were higher than Whites in Absorption in Daydreaming, Frightened Reactions to Daydreams, Positive Reactions to Daydreams (24-34 yr. olds only), Hallucinatory-Vividness of Daydreams, and content daydreaming scales of Problem-

Solving, Achievement-Oriented, Hostile, Heroic, and Guilt. In addition, for 17-23 yr. olds Present-Oriented Daydreams were higher for Blacks while for 24-34 year olds Present-Oriented Daydreams were higher for Whites. Black males had higher Interpersonal Curiosity than White males. Black males had lower Impersonal-Mechanical Curiosity than White males while the inverse was true for females.

II. Interrelations among daydreaming characteristics, health, and estrogen usage in pre-menopausal, menopausal, and post-menopausal women. A prior study of sex differences in daydreaming (see 1976-77 annual report) found a large drop in likelihood of sexual daydreaming in women at age 50 years as compared with women 35 to 49 years. Since age 50 is the menopausal transition period for most women, it was decided to investigate this potential relationship more closely. Information on daydreaming, menopausal state, health, demographic variables, and drug usage has been collected on 476 women 40 to 60 years of age. This information has been coded and punched. It is currently being analyzed statistically.

III. Vigilance and daydreaming incidence.
The vigilance task described earlier will be used to measure directly the incidence of daydreaming for people across the life-span. A life-span sample will be used since the self-reports of subjects suggests that with increasing age there is an increased ability to concentrate on any task without the intrusion of daydreams. A pilot study of ten subjects was carried out to test procedures. A series of studies is about to begin to determine the effects of total time in study, and of different rates of on and off time of stimuli.

IV. A Factor analysis of the items of the IPI
The 344 items of the IPI plus age were intercorrelated using the approximately 1400 people who completed it. This matrix was then subjected to a Principal Components (Factor) analysis. Fifty-three factors were then rotated to a Varimax Solution--these accounted for 58.8% of the correlation matrix variance. Of these 53 factors, 33 were found to have at least three items on them with loadings of .40 or greater. These criteria were used to reduce the number of items on a factor to a more homogeneous subset which lends itself to a meaningful interpretation and, because any factor with less than three items is psychometrically of little value. The 33 meaningfully interpretable factors could be put into nine categories: (a) attitudes toward self-revelation and daydreaming -- 2 factors, (b) aspects of daydreaming propensity/intensity -- 3 factors, (c) daydreaming affect -- 2 factors, (d) imagery in daydreams -- 2 factors, (e) content of daydreams -- 8 factors, (f) temporal perspective of daydreams -- 4 factors, (g) self-described attentional and mental activation characteristics -- 7 factors, (h) curiosity -- 3 factors, and (i) nightdreaming frequency -- 1 factor.

An alternate view of the results of this factor analysis comes from the factorial composition of the original 28 face valid scales of the IPI. Ten IPI scales were unifactorial, 14 scales were bifactorial, and four scales were multi-factorial. It is interesting to note that 77 of the 344 items of the IPI did not fall into any of the 33 meaningful factors.

The results of this factor analysis now permit the investigation of variables related to daydreaming and related mental activity in terms of uni-dimensional and orthogonal components. An additional advantage is the possibility of reducing the IPI length by 77 items.

V. A longitudinal study of daydreaming and related mental activity. Starting in January, 1979, men in the Baltimore Longitudinal Study of Aging have been completing the IPI. These men either have not taken the IPI before or have taken it 5-6 years ago. This data collection will continue until the entire BLSA population of men has been completely tested or retested. In addition 17-23 year old subjects are being recruited from the area colleges and universities. As women enter the study, they also will be tested and a limited number will be retested.

Significance to Bio-Medical Research and Program of the Institute: The study of daydreaming is fundamentally a study of thought processes. In order to understand fully the thought processes of man, the total spectrum of those processes needs to be examined. In addition, it is important to know how this wide spectrum is affected by aging. Thus the study of daydreaming in adults, along with other variables, such as differences in age, socio-economic status, attitudes, etc., may help us understand the fundamental processes which underlie all these behaviors.

Proposed Course of Project

A study of the dimensions of ongoing thought will be undertaken. This will involve the sampling of thoughts at random times during daily activity. Subjects will record the content and rate their thoughts along a number of dimensions. Subjects will consist of men and women from 30 years to 80 years of age. Outcomes will be compared with the results obtained by Eric Klinger on a 17-29 year old sample. Responses to the IPI have been obtained from Australia and these will be analyzed and compared to our American sample.

Publication:

Giambra, L. M. Sex differences in daydreaming and related mental activity from late teens to the early nineties. International Journal of Aging and Human Development, 1979-80, 10, 1-34.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00063-12 LBS

PERIOD COVERED
October 1, 1978 through September 30, 1979.

TITLE OF PROJECT (80 characters or less)

Learned Modification of Visceral Function in Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Joseph	Senior Staff Fellow	LBS, GRC, NIA
Other:	B. T. Engel	Chief, LBS	LBS, GRC, NIA
	S. P. Tzankoff	Senior Staff Fellow	CPB, GRC, NIA
	G. S. Roth	Research Chemist	CPB, GRC, NIA
	C. Filburn	Research Chemist	LMA, GRC, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Psychophysiology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.25	1.75	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to investigate the role of the central nervous system in behavior. In some experiments we examine the contribution of somatic factors in instrumental cardiovascular conditioning (ICC). Brain areas of special interest are the hippocampus, caudate nucleus and pyramidal tracts. Subjects are monkeys (Macaca mulatta). We also examine the quantitative and qualitative nature of the constraints that can be placed on the animal during ICC. Thus far, two procedures have been utilized. In the first, electrical stimulation is administered to localized CNS cardioacceleratory-pressor or cardiodeceleratory-depressor sites, e.g., hypothalamus. In a second procedure animals are trained to perform on a food-reinforced exercise task and then ability to control heart rate during combined exercise and ICC sessions is examined. In other experiments we examine age-related changes in pre- and post synaptic portions of nigrostriatal pathways using behavioral and biochemical analytical methods. The present topics include possible alterations in: development of post synaptic denervation hypersensitivity (b) specificity and affinity of dopamine receptor binding, (c) adenyl cyclase production and (d) synaptic release mechanisms that might occur with age.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: A-C. To determine the neural mechanisms involved in cardiovascular conditioning in monkeys.

D. To analyze the age differences in biochemical and structural characteristics of the brain in relation to behavior.

Methods Employed: A. In numerous experiments, in a variety of lower animals, the hippocampus, a neocortical structure, has been implicated in somatic control. This structure has a dominant frequency of 4-8 Hz which can be recorded from the pyramidal cell layer of the dorsal hippocampus when the animal performs or intends to perform a voluntary movement. This frequency has been designated as rhythmic slow activity (RSA). In attempting to deduce some of the possible CNS mechanisms that might be involved in the instrumental control of heart rate (HR), electrodes were chronically, bilaterally implanted in the hippocampi, pyramidal tracts and caudate nuclei of three monkeys operantly conditioned to speed and to slow HR. In one animal (M-3) the electrodes were implanted prior to training to assess RSA early in conditioning. Since the hippocampus seems to be related to movement, and since HR changes might be related to changes in movement, more RSA activity should be seen during HR speeding sessions than during slowing sessions. Each session comprised a 512 sec baseline period and a 2048 sec testing period. Three types of sessions were examined: "speeding sessions" during which the animal was operantly trained to speed its heart, "slowing sessions" during which the animal was trained to slow its heart, and no feedback (FB) sessions in which the animal was not required to speed or to slow its heart. It simply sat in the closed booth throughout a 512 sec baseline period and a 2048 sec "training" period. Information on speeding and slowing was provided by lights. A red light indicated that the animal was to slow its heart and a green light indicated a speeding condition. A white light functioned as a reinforcement light to indicate to the animal that it was performing correctly. A 10 ma, .45 sec electric shock was delivered to the tail once/8 sec for incorrect responding. For purposes of analysis the sessions were divided into 16 blocks of 128 sec duration and the baseline was divided into 4 blocks of 128 sec duration. Heart rate, systolic (SBP), and diastolic blood pressure (DBP) were recorded as well as samples of hippocampal EEG for the first 10 sec of each block. The "raw" hippocampal activity was filtered by a band pass filter over the 4 to 8 Hz range and then integrated. The activity in this range was defined as RSA and the amount of RSA for each 128 second block was computed. Additionally a fast Fourier analysis was carried out on the 10 sec samples of hippocampal activity so that the energy distribution from 1-50 Hz could be examined. Gross motor activity (M) was scored visually by a trained observer during the first 10 sec. of each 128 sec block.

B. There have been a great number of experiments in which electrical stimulation (ESB) has been delivered to various CNS structures and subsequent cardiovascular changes examined. However, most of these studies have been carried out in infraprimates. In the few studies which have been done in primates the animal has been anesthetized and unable to interact with its environment. Therefore, there was no chance of observing the possible interrelationships among ESB, cardiovascular changes and behavior of the animal. The present experiments were designed both to examine these relationships and to carry out a thorough mapping of the cardiovascular control areas in the infrahuman primate. Monkeys were trained to speed and slow HR as previously described (A). HR, SBP, and DBP were recorded (see A). Following training, an acrylic platform containing holes drilled to pre specified anterior-posterior and lateral coordinates corresponding to those of the anterior hypothalamus (AH), posterior hypothalamus (PH), subthalamus nucleus (SN), and the anterior commissure (AC), was stereotoxically mounted on the monkey's skull and cemented in place under aseptic conditions. Following a 1 week recovery period, the animal was given .5 mg/kg of phencyclidine and electrodes were then passed through the skull and lowered by hand to the pre determined area. Stimulation was delivered and the cardiovascular changes observed by examining BP and cardiotachometer tracings from the polygraph. When a suitable area was found, the BP and HR changes to the stimulation were noted. The lowest intensity was chosen that would produce a HR change of 10-20 BPM and comparable BP change. The electrode was then cemented in place. Dose response curves were then carried out in the fully awake animal and intensities were adjusted to produce a 10-20 BPM change in HR. Intensities usually ranged from 350 to 1000 ua depending upon the animal and the area being stimulated. Once they were established they were not changed. Cardiovascular responses were then examined during slowing (S), speeding (F), and $\overline{\text{FB}}$ sessions carried out as previously desribed (A) except that each segment and the baselines were shortened to 64 sec and 512 sec respectively. Stimulation for each session was delivered on alternate segments. Only one area was studied within a set of 3 sessions (S, F, $\overline{\text{FB}}$. At present only cardiovascular pressor areas have been examined.

C. In a related series of experiments we have been examining the constraints on instrumental cardiac conditioning (ICC) produced by physical exercise. In these experiments a monkey is trained to pull a lever attached to a weight box containing either 4 or 6 kg., depending upon the size of the animal, in order to receive a 300 mg food pellet. The monkeys were trained to receive a reinforcement on a fixed ratio schedule of reinforcement receiving 1 pellet for each 12 lever pulls (FR-12). HR, SBP, DBP, and number of lever pulls and O_2 consumption were measured during each session. Once stable exercise rates were achieved, each animal was trained as previously described (A) to slow its HR. HR, SBP, DBP, and O_2 consumption were measured during these sessions as well.

Once the monkeys were slowing their HRs reliably, the exercise and HR slowing sessions were combined so that the monkey had to pull the lever and slow its HR. The cardiovascular parameters previously mentioned were recorded.

D. It has been reported previously that a surgical lesion in the substantia nigra of young rats results in a substantial decline in the level of a dopamine (DA) in the caudate nucleus on the lesioned side. The caudate nucleus has been shown to be very important in the control of movement, and an imbalance in the neurotransmitters between the lesioned and unlesioned nigrostriatal pathways produces a directional dominance in an animal's movements such that after injection of certain drugs such as apomorphine (a DA receptor agonist) or amphetamine (which promotes the release of DA) animals can be induced to turn in circles. These rotations can be accurately measured with a device called a rotometer. Numerous studies have shown that: (a) amount of depletion; (b) DA receptor activity; (c) development of denervation receptor hypersensitivity; and (d) presynaptic release events can be gauged by the strength and directionality of the rotational behavior. This behavior seems to be almost exclusively modulated by the nigrostriatal system and by dopaminergic-cholinergic interactions within this system. In the present behavioral experiments lesions were produced unilaterally (left side) in the substantia nigras of young (4-6 mo) and old (25-29 mo) male and female rats following several prelesion baseline measures of rotational behavior. Following a 7-10 day recovery period baseline rotations were again examined to determine if there were any effects of surgery. After this the animals were given a graded series of doses of: (A) amphetamine (0.5, 1,2,5 mg/kg); (B) apomorphine (0.25,2,5 mg/kg); and (C) apomorphine (5mg/kg) followed by haloperidol (a dopamine receptor antagonist) (.25,.5,1,2 mg/kg). Each animal received all doses of all drugs (2 doses at each level), and it received the complete series of one drug before being given the next. In other experiments young and old animals were given amphetamine prior to lesioning to determine if a natural imbalance in dopamine exists between the left and right caudate nuclei and if this imbalance is enhanced or diminished with age. In a third set of experiments changes in the numbers of dopamine receptors in the caudate nuclei were assessed through binding studies carried out with tritiated haloperidol and an assessment also was made of adenyl cyclase, phosphodiesterase, and tyrosine hydroxylase in the caudate nuclei of young and old rats. In a fourth series of experiments the rotational behavioral method was used to examine what pharmacological agents might be utilized to overcome deficits in turning behavior seen in old animals. Old and young animals were given 1 mg/kg of amphetamine on alternate days (3 doses/wk) for 40-50 days after surgery. Very stable response rates were achieved with this method. They were then given 3 graded doses of L-DOPA alone (2,3,5 mg/kg) on alternate days. Then they were given 5 mg/kg dose of L-DOPA 1 hr. before a 1 mg/kg dose of amphetamine was given. They were tested for 1/2 hour. The L-DOPA-amphetamine combinations were replicated 3 times. Dopamine levels were assayed in the left and right striata from these animals to determine the efficacy of the lesion. A parallel group of lesioned old and young animals was used to assess striatal dopamine levels 1 hr. after an L-DOPA injection. Dopa decarboxylase and phosphodiesterase was assessed in still another group of old and young animals. Finally, development of post-synaptic hypersensitivity was investigated in lesioned young and old male and female rats in order to determine if differences existed in the proliferation of differential dopamine receptors as a function of age.

Major Findings: A. Each experimental period was divided into 16, 128 second blocks and difference scores for each dependent variable HR, SBP, DBP, RSA and M were computed by subtracting the mean score for each 128 sec. block (post block) from the score on the immediately preceding 128 sec. block (pre block) according to the following categories based upon HR: 1) speeding, blocks in which the animal increased its HR from $\geq$ 5 BPM to $\leq$10 BPM from the previous block (Hi criterion); 2) speeding, blocks in which the animal increased its HR $<$ 5 BPM (Lo criterion); 3) slowing, blocks in which the animal decreased its HR $<$-5 BPM (Lo criterion); 4) slowing, blocks in which the animal decreased its HR from $>$-5 BPM to $\leq$ -10 BPM (Hi criterion). In all cases the HR block selected had to be above (speeding) or below (slowing) baseline in order to be considered. Blocks were selected from the $\overline{\text{FB}}$ sessions in a similar manner. Limits of 10 BPM were placed on the HR change so that the effects of feedback (FB) conditions could be examined on RSA and M while limiting the overall variability of the HR changes. Results showed that the differences between slow and fast conditions were not any greater under FB than $\overline{\text{FB}}$ conditions, thus the HR changaes were essentially the same under both conditions. Analyses of variance revealed that RSA activity was greater during speeding than during slowing for both FB and $\overline{\text{FB}}$ for the three animals (M-1, F=8.79 df=1/939 $p<.01$; M-2, F=5.37 df 1/429 $p<.01$; M-3, F=148.22 df=1/823 $p<.01$). However, this analysis also revealed that the decrease in RSA activity under $\overline{\text{FB}}$ slowing is much greater than that seen under FB slowing producing greater differences in RSA between $\overline{\text{FB}}$ slowing and speeding than under FB. This factor contributed to statistically significant FB condition by direction of HR hange condition interactions seen in two of the animals (M-1 F=6.78, df=1/929 $p<.01$; M-2, F=4.93 df=1/429 $p<.05$). In the third monkey (M-3) the interaction approached statistical significance (F=3.58 df=1/823 $p<.055$). Further analyses revealed that these findings were a function of the degree of HR slowing or speeding that the animal was exhibiting--that is whether or not Hi cardiac (>5-10 BPM) or low cardiac ($\leq$5 BPM) changes were being exhibited. RSA changes were greater under the Hi criterion condition. Interestingly RSA activity was much smaller under the Hi criterion slowing $\overline{\text{FB}}$ condition than under the Hi criteria slowing FB condition and these differences were found to be statistically significant in two monkeys (M-1, t=3.1 df=70 $p<.01$, M-2, 5=2.12 df=34 $p<.05$). Thus, the largest amount of RSA is seen during HR speeding regardless of whether the animal is under FB or $\overline{\text{FB}}$ conditions and significantly more RSA is seen under HR slowing under FB conditions than under $\overline{\text{FB}}$ conditions, especially in blocks where the animal is exhibiting a Hi degree of HR slowing.

In contrast to RSA changes, M changes tended to be small and higher during HR speeding than HR slowing, however, there was no differential effect of FB on M for any of the animals. SBP and DBP changes were small and variable during both FB and $\overline{\text{FB}}$ conditions. Both SBP and DBP were higher during HR speeding than during HR slowing but tended to show overall differential directionality among the three animals between FB and $\overline{\text{FB}}$ conditions and failed to show the consistency that had been seen in other parameters (e.g., RSA) under similar conditions. We conclude that changes in somatic activity do not contribute a great deal to the animal's ability to perform during ICC. This suggests that the cardiac-somatic link may not be operating solely and at all times during conditioning, and further suggests that other factors may be operating as well. One of these, as shown by the RSA data, may be attention. RSA activity has been found by several investigators to increase when the animal is attending or orienting to discriminitive stimuli. In the present experiment there were discriminative stimuli present during FB but not $\overline{FB}$. It was apparent that while RSA and M were similar during HR speeding, there were decided differences during HR slowing especially under $\overline{FB}$. We believe that the attentional component of RSA activity during HR speeding is masked by the somatic component. However, during FB slowing the attentional component becomes unmasked since RSA does not decline as in the $\overline{FB}$ condition. These data suggest that there is an attentional component in the mediation of these HR changes during FB. These relationships between RSA and HR seem to be highly stable throughout conditioning.

Data from the 10 sec samples from the hippocampus, pyramidal tracts and caudate nuclei have not yet been analyzed.

B. The effects of ESB on HR during S. F. and $\overline{FB}$ sessions have been partially analyzed in one monkey from 4 brain areas i.e., the posterior hypothalamus (PH), anterior hypothalamus (AH) anterior commisure (AC) and subthalamic nucleus (SN). In a second monkey stimulation data from the AH and SN have not been completed, but HR data collection from the AC and PH have been completed and analyzed. For these determinations, HR difference scores were computed by subtracting the HR for each stimulation segment (even segments) and non-stimulation segments (odd segments) from the baseline HR for each type of session (F,S,$\overline{FB}$). The means and standard errors of the mean (sem) of these analyses are given below for the PH and AC from monkeys 1 and 2.

	PH			AC		
	$\overline{FB}$	F	S	$\overline{FB}$	F	S
M-1						
STIM	23.1 ± 2.5	19.3 ± 2.8	1.8 ± 1.1	13.2 ± 1.9	21.1 ± 3.2	-8.9 ± 6.8
NO STIM	15.7 ± 4.2	10.3 ± 1.1	-2.7 ± 0.8	2.2 ± 1.7	3.7 ± 2.0	-10.3 ± 3.5
M-2						
STIM	6.9 ± 1.5	6.4 ± 2.2	-0.9 ± 0.3	12.4 ± 2.0	7.3 ± 1.2	-7.9 ± 1.8
NO STIM	1.9 ± 1.1	.7 ± 1.7	-13.5 ± 1.5	2.7 ± 1.2	2.7 ± 1.2	-16.8 ± 1.3

Several points can be made upon examination of this table,

1. HR response from both animals tended to be highly similar from the two areas both for stimulation and non stimulation periods.

2. The ordering of the responses across the $\overline{FB}$, F, and S conditions was similar for both animals.

3. Generally, the stimulus was effective in producing increases in HR in both animals across the FB, F, S conditions (e.g., M-1, AC FB NON-STIM vs. STIM $t=4.31$ $df=94$ $p<.001$; M-2, $t=2.64$ $df=158$ $p<.01$; M-1, PH F NON-STIM vs. STIM $t=9.1$ $df=136$ $p<.001$; M-3, $t=1.83$ $df=110$ $p<.06$; M-2, PH S NON-STIM vs. STIM $t=4.37$ $df=110$ $p<.001$).

4. Under the S condition HR increases during stimulation were attenuated relative to the FB and F conditions for both animals (e.g., M-1, F vs. S PH $t=8.80$ $df=136$ $p<.001$; M-3, $t=2.22$ $df=110$ $p<.05$; AC M-1, F vs. S $t=3.94$ $df=86$ $p<.001$; M-3, $t=6.22$ $df=112$ $p<.001$). Animal 1 showed this attenuation for stimulus relative to non stimulus blocks during slowing as well. Note the small differences in HR for this animal in column 3 of the table.

These findings suggest that during HR slowing sessions the animals were able to lower their HRs in the face of ESB of the same magnitude as that given during the F and FB conditions. There was no indication that the effects of brain stimulation operate in an additive manner to aid in raising HR during the F condition in those brain areas since an examination of the table reveals only very small differences between the HR changes under the F and FB conditions for either monkey during stimulation especially in the posterior hypothalamus. It would appear that as long as no constraints are placed on the animal for speeding as in the F and FB conditons, the animal does not attenuate its HR; however, if constraints are placed on HR during the S condition, the animal can block or ameliorate the stimulation effects. It is also to be noted that these effects are not the spurious result of volume conduction of the ESB since stimulation of two other brain areas (SN and AH) in M-2 produced different patterns of effects in each area. These are illustrated in the table below.

	AH			SN		
M-2	FB	F	S	FB	F	S
STIM	10.7 ± 1.6	8.9 ± 1.6	5.5 ± .70	1.6 ± 0.9	8.2 ± 2.5	-2.8 ± 1.5
NON STIM	2.3 ± 1.6	1.3 ± 1.4	-6.9 ± 1.4	-0.8 ± 1.4	3.1 ± 2.0	-7.8 ± 1.2

Note that in the AH the animal was not able to attenuate the effects of ESB during slowing. This can be seen by comparing the average change in HR for slowing under stimulation with those under the F and FB conditions. On the other hand, with stimulation in the SN the animal attenuated the stimulus under both S and FB conditions but did not under the F condition, suggesting that ESB at this site might be aversive, and that the animal will try to negate its effects under conditions where there is no penalty for doing so. Thus, there is differential, area dependent responses in 3 out of 4 areas examined. We are currently carrying out the analyses of the BP data and examining other CNS sites to determine the pathways involved in these CV-behavioral interactions.

C. We have just begun the phase of the experiment in which the animals are placed in combined exercise and slowing sessions. Analyses of the very tentative data from these experiments have shown that the animals are learning to control their HR during exercise. It is to be noted that only the HR and number of bar pulls have been analyzed thus far. For this analysis the HR difference score was obtained by subtracting the baseline HR for each session for exercise alone and exercise + FB sessions. For monkey 1 four combined and four non-combined sessions were analyzed while for monkey 2 four combined and five non-combined sessions were analyzed. The bar pulls (P) were analyzed per 64 sec. The means for bar pulls and HR change for the combined and uncombined sessions are shown in the following table.

	UNCOMBINED				COMBINED			
	HR		P/64 sec		HR		P/64 sec	
	$\bar{X}$	sem	$\bar{X}$	sem	$\bar{X}$	sem	$\bar{X}$	sem
M-1	88.54	± 1.80	38.03	± 1.42	77.91	± 2.48	46.13	± 1.94
M-2	69.96	± 2.64	10.64	± 1.18	54.94	± 1.74	50.05	± 2.01

It can be seen that the HR change from baseline was lower for both animals during the combined sessions (M-1, $t=3.45$ $p< .001$ $df=123$; M-2 $t=7.94$ $df=142$ $p< .001$). Thus the monkeys were able to both attenuate their HR increases while increasing their bar pulls during the combined sessions. While any conclusions that can be drawn from this preliminary analysis are very tentative, the data suggest that under the present training conditions the monkey can lower its HR while exercising under a contingent slow-exercise condition.

D. Since all animals (32; 8 old males, 8 young males, 9 old females, 7 young females) were lesioned in the left substantia nigra, DA levels in the left caudate nucleus should be diminished. When amphetamine is given, DA release is promoted on the unlesioned side and the animal turns toward the left. It has been shown in young animals that the greater the strength of turning toward the lesioned side the greater the imbalance in DA that exists between the lesioned and unlesioned striatum. Results of the analysis of baseline turning rates showed that old animals emitted less overall turning than did young animals ($\bar{X}$ = 2.0 turns/30 min, old; $\bar{X}$ = 16.5 turns/30 min, young). Under the influence of amphetamine the animals began to turn increasingly toward the left as a function of dose. This occurred in both old and young animals. A direct comparison of the magnitude of the differences in left turn behavior with increasing doses of amphetamine was difficult to make because there were age differences in the initial turning behavior prior to the drug injections. Therefore, the data were transformed and a strength of response measure was computed by taking the left turns for each animal and dividing them by the right turns. Baselines were equated (old .88, young .85) and the resulting analysis of variance showed that old animals emitted lower ratios of left/right turns, and that this was true throughout the dose range ($\bar{X}$ dose ratio overall: Old = 5.5, young = 24.0). There were no differences between males and females in response strength. Old animals tended to increase both right and left turns as the dose of amphetamine was increased, while young animals selectively increased left turns. These findings suggest that there is a defect in DA responsivity at either the pre or post synaptic receptor on the unlesioned side since histological analysis of the brain of these animals showed that the lesions were all of the same size. It is difficult to say where the deficit lies, but on the basis of the apomorphine data discussed below it may be presynaptic. Since the caudate nucleus on the lesioned side develops degeneration hypersensitivity, apomorphine a DA receptor agonist should affect it more strongly, and the animal should exhibit contralateral turning (i.e., away from the lesioned side) behavior. Following the administration of this agonist both young and old rats exhibited turning behavior and no differences as a function of age were seen ($F<1.0$).

Further evidence for this proposed deficit in presynaptic mediation of rotational behavior can be found from data in our laboratory in which L-DOPA potentiation of amphetamine induced turning occurred in young rats but not in the senescent rat. Young rats increased their left/right turn ratios by 50% while old rats showed a 23% decline in response ratios. Subsequent analyses of the DA levels from the lesioned and unlesioned striata showed no differences between old and young animals ($\bar{X}$ young=12.16 $\pm$ .854, $\bar{X}$ old=19.99 $\pm$ 3.20 ug/g - right striatum; $\bar{X}$ young=.473 $\pm$ .163, $\bar{X}$ old=.990 $\pm$ .411 ug/g left striatum) in DA levels. When L-DOPA was given to 1/2 of the groups of lesioned young and old animals prior to sacrifice, the DA levels in both groups of unlesioned striata were higher than in the untreated groups ($\bar{X}$ young=25.22 $\pm$ .616, $\bar{X}$ old=19.67 $\pm$ 3.01), but there were greater differences in DA levels in the young L-DOPA and non L-DOPA injected groups than in the old L-DOPA and nonL-DOPA injected groups ($t=14.32$ $df=15$ young; $t=1.12$ $df=11$ $p > .05$ old) indicating some loss of ability to synthetize DA in the old animals. However, DA levels in the unlesioned striata of both the old and young animals were extremely high relative to the lesioned striata in these groups. These results suggest that there is not a deficit in synthesis, *per se*, producing the loss of L-DOPA potentiation of amphetamine induced turning in the senescent animals. This is further supported by subsequent biochemical analyses of tyrosine hydroxylase and dopa decarboxylase, two enzymes important in the synthesis of dopamine. Neither enzyme was found to be depleted to any appreciable degree in the old animal. Thus the deficit may be in release or metabolism of DA in the old animal. There is also some indication of receptor involvement in the non-supersensitive striata since a DA receptor binding assay with haloperidol indicates a 33% loss in DA receptor number with senescence. However, there does not appear to be a significant decline in DA stimulated cyclase activity after 12 mo. in the male rats. Since we have not found any deficits in turning behavior in 10 rats of this age, this finding suggests that there is an involvement of only one type of dopamine receptor in this response. This receptor is more easily assessed with the 3H-haloperidol binding assay. We are currently carrying out experiments to determine if the DA receptor proliferation observed with the develpment of degeneration hypersensitivity declines with age. The apomorphine data would suggest that it does not, but biochemical confirmation is needed. Thus, although more experiments are needed, we believe that the rotational response deficit seen in the old animal may be associated with the release or metabolism of DA, and may further be associated with a decline of DA receptors in the non-hypersensitive (i.e., non lesioned striatum).

Significance to Bio-medical Research and the Program of the Institute: There is growing evidence that the central nervous system provides a high degree of plasticity for peripheral motor functions. THus, responses which ordinarily occur in one way under one set of conditions, may occur in another way under another set of conditions. Two processes which operate upon the nervous system to modulate peripheral function are learning and aging. Until we understand fully how these processes operate, and under what conditions they will express themselves, we will never be able to explain behavior in normal or diseased individuals, or in subjects of any age. Furthermore, when we understand the principles which underlie neurally mediated plasticity, we should be able to evolve strategies which will enable subjects to compensate, at least in part, for the infirmities associated with the disorders of adult life. For example, the neural systems that we are now studying in the primate project deal with the integration of somato-motor and cardiovascular responses. It is well-known that both of these motor systems become less adaptable with age; but what is not known is the extent to which this diminished adaptability can be modified by experience. Moreover, we believe that the experiments involving the interaction of ESB and behavior during ICC, and the experiments concerned with instrumental modification of HR during exercise lend themselves well to delineation of the contribution that experience makes in modifying neural events. Another example of the relevance of this project to the assessment of age-dependent neural changes in plasticity comes from the work on the nigrostriatal system. It is well-known that neuromuscular disorders of late adult life such as Parkinson's Disease are associated with various changes in function and structure of the nigrostriatal system. It is our goal to determine the extent to which these age-related declines can be ameliorated.

Proposed Course of the Project: A,B,C. The present experiments will be continued so that the contributions of the hippocampus during instrumental heart rate conditioning can be assessed more definitively. Other CNS areas concerned with somatic control will be examined during cardiovascular conditioning in order to determine their involvement in mediating this response. In conjunction with this, data collection is continuing on the effects of stimulation of various anterior hypothalamic, thalamic, commissural and posterior hypothalamic pressor, depressor cardioacceleratory and deceleratory areas during the three training procedures , i.e., fast, slow and no feedback, in order to further characterize the effects thus far observed. In later experiments attempts will be made to place electrodes more posteriorly in brain stem sites to determine if stimulation of these areas can be altered during ICC. The exercise experiments are also progressing. The animals will be taught to speed their HR during exercise and the combined effect of these conditions observed. Flow probes will be placed around the aortas of these animals so that cardiac stroke volume as well as the other measures (HR, etc.) can be taken during combined and uncombined conditioning and exercise sessions.

D. So far the project has concentrated on the dopamine system. Further investigations will examine turning behavior after modifications on serotonergic, noradrenergic, and cholinergic systems. In addition, control studies will be carried out to determine other factors that might account for the differences in turning behavior with age (e.g., differences in the rate of metabolizing test drugs, etc.). Finally, we will begin to analyze the effects of intracerebral administration of the test drugs on rotation in young and old animals.

Publications:

Joseph, J. A., Berger, R. E., Engel, B. T. and Roth, G. S.: Age-related changes in the nigrostriatum: A behavioral and biochemical analysis. J. Gerontol., 33: 643-649, 1978.

Gottlieb, S. H. and Engel, B. T. Autonomic interactions in the control of heart rate in the monkey. Psychophysiol., in press.

Joseph, J. A. and Engel, B. T. Cardiovascular response deficits in the senescent organism: Importance of central neural and cardiovascular interactions. In Weisfeldt, M. A. (Ed.): The Senescent Heart, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00064-18 LBS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Problem Solving and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIs:	David Arenberg,	Chief, Learning & Problem Solving Section	LBS GRC NIA
	Leonard M. Giambra	Research Psychologist	LBS GRC NIA
Others:	None		

COOPERATING UNITS (if any)
Baltimore City Hospitals

LAB/BRANCH
Gerontology Research Center - LBS

SECTION
Learning and Problem Solving Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	.6	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary purposes of this project on aging are to; (1) identify reasoning processes; (2) determine how these processes change with age; and (3) develop techniques for reducing age deficits in reasoning performance. Reasoning is studied using problem solving procedures including concept learning and concept identification.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The general goals are to explore and identify reasoning processes in man, to determine in what ways these processes change with age, and to develop techniques for reducing age deficits in reasoning performance. In this project, reasoning is studied by using problem-solving procedures in which on-going solution behavior can be observed and quantified. Experiments are designed to answer such questions as: (1) Is effectiveness in acquiring relevant information affected by aging? (2) Is effectiveness in synthesizing available information affected by aging? (3) What kinds of solution strategies are used and in what ways are they related to age? (4) How does imposing a memory load affect solution strategies for young and old adults?

Methods Employed: All of the three experiments in progress are long-term aging studies. Experiments IV and V are longitudinal studies; and in Experiment XI each subject solves more than 100 problems and a model is constructed which describes and predicts how he or she solves a wide variety of concept-learning problems.

Experiment V is a longitudinal study of concept identification. In this study, subjects select instances (examples) sequentially and are informed whether each selection is a positive example of the concept or a negative example. On the basis of the information elicited by these selections and their classification, the specific attributes of the concept can be identified. This procedure provides a measure of information gain for each selection. Ordinarily when subjects select instances, logically equivalent selections do not always result in equivalent information gains, and fortuitous selections can alter drastically the difficulty of a problem. A technique was developed which provides equivalent information gains for logically equivalent selections and avoids fortuitous gains in information. This technique also has two other important characteristics: (1) different types of problems which are logically equivalent can be constructed and compared, e.g., conjunctive concepts in which two attributes must both be present can be matched with two-attribute disjunctive concepts in which either attribute can be present; and (2) initial information gain can be manipulated because the experimenter can minimize or maximize the information gain for each selection. Experiment V was designed to study age differences and age changes for six different types of problems determined by the number of attributes in the concept (one or two), and for two-attribute problems, the concept rule (conjunctive and disjunctive) and the amount of initial information gain.

Experiment XI is a concept study in which each subject solves a large number of problems. The literature in concept identification is based almost entirely on mean effects of subsamples in which each person solves one or a few problems. It has not been established that the variables which affect the mean performance of groups would affect an individual in the same way. When a person solves one or a few problems, his approach to reasoning can be characterized as unstable, variable, transient, and highly subject to chance occurrences. Solving many problems is expected to result in a steady state of performance. In that steady state, strategies can be more readily elicited and the effects of various independent variables on changes in strategy and on other performance measures can be studied. After solving 48 complete learning problems, and again after 96, 8 more problems are solved in which subjects "think out loud" throughout each problem. When individuals "think out loud" they are verbalizing all the thoughts about the concept problem that are in their conscious awareness. They are specifically asked: (a) to relate the basis of their concept classification of the stimulus instance before them; (b) to tell if they had seen the stimulus instance earlier in the problem and if they remember its concept classification; (c) to relate how they use the feedback information about the correct classification of the stimulus instance before them to help them learn the concept; and (d) for any mnemonics they use to remember any and all aspects of a problem during the solution. The protocols of these "thinking-out-loud" problems constitute the primary data of this study. They are used to construct individual models of how a subject solves complex concept problems with which he is highly practiced. These models have as a general goal the prediction of each individual's performance on concept learning problems by specifying at each point in a problem how the individual will classify a stimulus instance as an exemplar or a nonexemplar of the concept. This general goal is met by succeeding at the more fundamental goals of specifying: (a) what information and past stimulus instances are stored in memory as well as for how long and what the retrieval cues are; and (b) how information on the exemplar status of a stimulus instance is utilized, inductively or deductively, to arrive at a solution to the problem, i.e., to identify the unknown concept. To achieve goals (a) and (b) a detailed specification of mechanisms is needed. These finely detailed mechanisms, appropriately connected in a flow diagram, constitute the individual's model. Comparisons of these mechanisms and their connections provide the means of specifying individual differences as well as age (generational) differences.

Major Findings: Experiment V is the longitudinal study of concept problem solving and age. Previous longitudinal analyses of total number of problems solved correctly showed that, although for the entire sample the mean did not decline during a seven year interval, the magnitude of change was inversely related to age; i.e., the younger men tended to improve somewhat whereas the older men tended to decline. However, in order to be included in those analyses, it was necessary that a participant attempt to solve all twelve problems at both the first and the second time of measurement. That requirement is likely to bias (positively) the results, particularly for the older groups; i.e., the better problem solvers tend to be over-represented because the poorer performers tend to be excluded. Unbiased estimates of change can be determined by comparing first-time measures of men born during the same period but measured at different times. Regression procedures developed in this Section to analyze comparisons within birth cohorts were applied to first-time measures for eight cohorts ($\underline{N} = 673$). The largest estimates of decline were found for the two earliest born (oldest) cohorts. These data support the longitudinal analyses indicating that decline in solving reasoning problems occurs late in life.

Since Experiment XI represents an individualistic approach to the study of concept learning, it would be fruitful to determine the extent to which the individuals may be different from one another. It is a reasonable, though not necessary, inference that the individuals who show a lack of similarity as indicated by performance measures should also be dissimilar in their individual models.

On each concept learning problem, four performance measures were obtained: (a) number of errors made in guessing the exemplar status of an instance while solving the concept problem; (b) the trial number on which the last error was made; (c) the time taken to go through the 40 trials (instances) of the problem; and (d) the number of universe classification errors (after the solution part of the problem is completed, subjects are asked to guess the exemplar status of each of the 27 different possible stimulus instances in the universe, and no feedback is given). These four performance measures were obtained for each of the 64 problems comprising blocks 1-6, 8, and 9 for each of the 22 subjects included in this study thus far. A mean was then obtained for each of the eight blocks for each performance measure. Thus each subject had four performance measures on each of eight problem blocks. These measures were then converted to standard scores so that each measure had the same mean and variance. The 32 standardized scores for each individual were used in an hierarchical cluster analysis which progressively placed in appropriate clusters each of the 22 subjects. Thus at any point in the hierarchical analysis one could determine which subjects were similar and which were different. As the analysis progresses to fewer and fewer clusters, the criterion of similarity becomes weaker and weaker until everyone is put in a single cluster.

Using specific criteria it was determined that eight clusters or patterns of performance were evident in the 22 subjects. These eight patterns could, with two exceptions, be ranked as to effectiveness of performance. The cluster representing the subjects with the best performance consisted of both 18 year olds and the 20, 40, and 66 year old subjects. The next best performance cluster consisted of the 48, 72, and 74 year old subjects. The third best performance cluster had one 26 year old and the 35 and 54 year old subjects. The next best cluster consisted of the other 26 year old and the 12, 13, and 51 year old subjects. The poorest two clusters consisted of, respectively, the 81 and 87 year old subjects and the 89, 96, and 106 year old subjects. Two subjects, a 63 year old male and a 90 year old female, each formed a single member cluster which was not easily ranked as to level of performance. The 63 year old mean block standardized scores on all three error performance measures were close in magnitude to the measure of second best performance cluster consisting of the 48, 72, and 74 year old subjects; but his mean block time measure was more than two standardized units slower than that group. The 90 year old mean block standardized scores for errors were similar to that of the 4th best performance group consisting of the 12, 13, 26, and 51 year old subjects. However, her mean block time measure was also more than 2 standardized units slower than that group. Thus the 63 and 90 year old subjects were able to trade increased time for increased accuracy. Overall, one can only say that the late teens and early 20's do well and the late nineties do poorly. The intermediate ages form no consistent age orderings. The 90 year old female shows that some quite old subjects can trade time (speed) for accuracy, but for most over 80 this was not what occurred. It is hoped that the individual models to be obtained for these subjects will provide an explanation of these cluster groupings.

Significance to Bio-Medical Research and the Program of the Institute:
Reasoning is among the most prized behaviors of man and among the most elusive for experimental study. In this project, methods have been and will be developed to obtain quantifiable measures of step-by-step performance on reasoning problems. Some of these methods also provide patterns of response which represent strategies in solving such problems.

Measures are obtained in current experiments to study changes in reasoning processes with age. These studies, in addition to identifying basic reasoning processes, should indicate the pervasiveness of reasoning deficits with age, whether education and cognitive activity mitigate such deficits, and what techniques could be used to minimize decline in reasoning.

Proposed Course of Project: The longitudinal study of concept problem solving (Experiment V) will be replicated with women; data collection will begin immediately. Individual model building will continue for Experiment XI. New concept learning studies will include: (a) time of each trial as an additional variable; (b) requiring a criterion of solution to be reached (currently a fixed number of trials is used); and (c) using one dimensional (rather than two) problems with auditory presentation (rather than visual).

Publication:
Arenberg, D. Learning from our mistakes in aging research. Experimental Aging Research, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00065-19 LBS

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Verbal Learning and Age

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David Arenberg	Chief, Learning & Problem Solving Section	LBS GRC NIA

Others: None

COOPERATING UNITS (if any)

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Elizabeth A. Robertson-Tchabo	University of Maryland
Larry Potash	

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Learning and Problem Solving

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	.6	2.7

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary purposes of this project on aging are to identify measures of verbal learning and memory which change with age, to specify psychological processes and their relationships with age, and to develop procedures for improving learning and memory performance in the elderly.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Primary objectives are: (1) to identify which aspects of learning and memory change with age (and which do not); (2) to specify psychological processes underlying such age changes; (3) to identify health, nutrition, biochemical, and personality variables which are correlated with performance or with change in performance; and (4) to develop procedures to improve learning and memory performance in the elderly.

Methods Employed: Experiment XXXIII is a recently initiated longitudinal study which includes immediate free recall, delayed memory, dichotic listening, forward digit span, simple reaction time, and four levels of choice reaction time. These procedures (somewhat modified) were included in Experiment XXXI of this project. In immediate free recall, twelve familiar words are presented visually at a rate of one per second, and the task is to recall as many words as possible (in any order and without time limits) at the end of the presentation. Two delayed memory procedures are included. In delayed recall, the task is to recall as many words of the free-recall list as possible after an interpolated task (digit span). In delayed recognition, the twelve words in a free-recall list mixed with 12 other familiar words are presented in random order after the interpolated task; the recognition task is to identify which words are from the list and which are not. In dichotic listening, two different digits are presented simultaneously, one to each ear; the task is to identify the two stimuli. In forward digit span, lists of three to nine digits are presented at a rate of one digit per second, and the task is to respond with all the digits in a list in the order presented. In simple reaction time, a zero appears on a screen 15 times during a 90 second period, and the task is to press a button as fast as possible for each stimulus. In the four choice reaction procedures, 90 digits appear at a rate of one per second, and 15 of these digits are target stimuli which are signals to press the button as fast as possible. The four rules for responding are: (1) a specific digit; (2) any odd (or even) digit; (3) any even digit which immediately follows an odd digit (or vice versa); and (4) any even digit which immediately follows an even digit, and any odd digit which immediately follows an odd digit. Current analyses are cross-sectional correlations between performance and age for 81 men.

Experiment XXXV is an age study of free recall in which some items (words) in a list are presented two or three times. For some words, the repetitions are contiguous (massed); for others, the repetitions are separated by several other words (distributed). Previous research has demonstrated that for young adults words with distributed repetitions are more likely to be recalled than words with massed repetitions. Animal studies in this Laboratory, however, have demonstrated that complex maze learning is enhanced for old rats (but not for young rats) when learning trials are massed. The current study is designed to explore whether massed practice (in the sense of repetitions of items) is beneficial for recall memory in old adults. Each word is shown for 2 seconds, but the interval between words is varied (1 or 7 seconds) to determine whether time to assimilate the material to be recalled affects the "distribution-of-practice" effects. The two age groups are: (1) 18-25 years and (2) 60-75 years.

Major Findings: Experiment XXXIII is the longitudinal study of cognitive and response time measures originally included in a previous cross-sectional study of healthy men who were taking no medication (Experiment XXXI). Preliminary cross-sectional correlations with age were comparable with the earlier results. Each measure was repeated; therefore there are two correlations for each: immediate free recall-- -.54 and -.52; delayed recall-- -.34 and -.50; delayed recognition (hits)-- -.17 and -.22; dichotic pairs-- -.31 and -.33; digit span-- -.35 and -.38; simple reaction time-- .34 and .29; choice reaction time 1 (CRT-1)-- .41 and .37; CRT-2-- .23 and .20; CRT-3-- .30 and .28; CRT-4-- .10 and .13; CRT-3, errors-- -.25 and -.11; CRT-4, errors-- -.31 and -.39.

Experiment XXXV is the free-recall study in which repetitions of items are contiguous (massed practice) or separated by several other items (distributed practice). The hypothesis that the elderly would benefit from massed practice was not confirmed. In fact, at both the slow and the fast pace, the elderly recalled more distributed items than massed items--similar to the effects of distribution of items found for young adults in previous studies.

Significance to Bio-Medical Research and the Program of the Institute: Memory and learning are central to experimental psychology, and some of the most striking and consistently reported behavioral age differences in the gerontological literature have been found in verbal learning performance. The experiments in this project are designed to identify basic mechanisms of learning and retention and to measure differences and changes in these functions that occur with age. In addition, knowledge about experimental variables which affect age differences will be valuable in developing techniques for optimizing learning and memory of the older person.

Proposed Course of Project: Massed practice, which was previously shown to be a favorable condition for old rats to learn a complex maze, will be applied to other human learning studies. The study (Experiment XXXV) in which this procedure was used with elderly people was designed to prevent rehearsal between trials. Additional studies may include procedures which allow rehearsal, e.g., paired-associate learning. If feasible, the age study (Experiment XXVIII) of the effects of ethanol on memory and information processing will be replicated except that the ethanol will be ingested rather than infused. The earlier study showed for some measures that ethanol was particularly detrimental to performance of the old. If these findings are replicated when the ethanol is ingested, they will have important social consequences.

Publications:

Arenberg, D. Introduction to a symposium: Toward comprehensive intervention programs for memory problems among the aged. *Experimental Aging Research*, 1978, *4*, 233.

Poon, L. W., Fozard, J. L., Cermak, L. S., Arenberg, D., & Thompson, L. W. (Eds.). *New directions in memory and aging: Proceedings of the George Talland memorial conference*. Hillsdale, N.J.: Erlbaum, in press.

Arenberg, D. Comments on the processes which account for memory declines with age. In L. W. Poon, J. L. Fozard, L. S. Cermak, D. Arenberg, & L. W. Thompson (Eds.). New directions in memory and aging: Proceedings of the George Talland memorial conference. Hillsdale, N. J.: Erlbaum, in press.

Arenberg, D. Localization of decline and the role of attention in memory. L. W. Poon, J. L. Fozard, L. S. Cermak, D. Arenberg, & L. W. Thompson (Eds.). New directions in memory and aging: Proceedings of the George Talland memorial conference. Hillsdale, N.J.: Erlbaum, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00066-18 LBS

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Perceptual Retention and Age

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David Arenberg	Chief, Learning and Problem Solving Section	LBS GRC NIA
OTHER:	Paul T. Costa, Jr.	Chief, Stress & Coping Section	LBS GRC NIA
	Robert R. McCrae	Stress & Coping Section	LBS GRC NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

Elizabeth A. Robertson-Tchabo, University of Maryland

Don Reynolds

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Learning and Problem Solving

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	.8	.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary purposes of this project on aging are: (1) to investigate perceptual retention and interference; (2) to determine under what conditions age differences in retention are affected by interference; and (3) to investigate processes of interference and perception. Current studies include nonverbal memory, a visual analog of the dichotic-listening procedure, and massed vs. distributed practice in simple reaction time and decision time.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: One general objective is to investigate the effects of interference in perceptual retention and in perception: (1) to determine whether aging results in increased susceptibility to interference; (2) to explore conditions which affect age differences in interference; and (3) to develop procedures for testing mechanisms which may account for the empirical findings. Another objective is to study non-verbal memory and the conditions which improve such memory, especially for the old.

Methods Employed: Experiment VII is a longitudinal study of memory for designs in which subjects attempt to reproduce visual designs from memory. The Benton Visual Retention Test is used, and the primary dependent measure is the total number of errors in all ten designs. Each design consists of geometric figures presented for ten seconds and then withdrawn. The task is to reproduce each design from memory. Subjects may take as much time as they need to draw the design. The current analyses include: (1) the relationship of the ten scales of the Guilford-Zimmerman Temperament Survey with longitudinal change in performance on the Benton over six years for 52 men initially 70 or older; and (2) a preliminary cross-sectional analysis of Benton performance for the women's sample ($N = 129$).

Experiment IX is a longitudinal study of personality based on data from the Guilford-Zimmerman Temperament Survey (GZTS). The GZTS is a questionnaire which measures ten personality traits. Data collection began in 1958 and is continuing at the present time. Current analyses are based on data collected between 1958 and 1978. The primary purposes of these analyses were to determine the stabilities of the ten scales of the GZTS over six years and over twelve years for three age groups of men (17-44, 45-59, and 60-85 at time of initial measures). There were 460 men included in the analyses of six-year data, and 222 men for twelve-year data.

Experiment X is a study of visual information processing using a visual analog of the dichotic-listening procedure. Verbal information is generally believed to be processed sequentially by the auditory system and simultaneously by the visual system. The dichotic-listening procedure, in which pairs of different stimuli are presented simultaneously (one to each ear) and two or more pairs are presented sequentially, has been used to demonstrate that auditory information is processed sequentially. Under specific timing conditions, many people report such stimuli ear by ear rather than by simultaneous pairs (as presented). A visual analog of the dichotic-listening procedure was devised in this Section to determine whether, under specific timing conditions, visually presented stimuli are reported sequentially (visual field by visual field) rather than by simultaneous pairs. Each simultaneous pair consists of two different digits, one in the left visual field and one in the right.

Experiment XI is an age study of massed vs. distributed practice in simple reaction time and decision time. Animal studies in this laboratory have shown that old rats (unlike young mature rats) learn a complex maze with fewer errors when trials are close together in time (massed) than when trials are several hours (or a day) apart. Experiment XI is designed to determine whether massed practice is beneficial to old humans on response-time tasks which are expected to show improvement in performance over trials. Two tasks are used: (1) simple reaction time -- during a 90 sec. period, a zero appears 15 times on a screen, and the subject presses a hand-held button as quickly as he can; and (2) choice reaction time (decision time) -- 90 digits are presented sequentially on a screen at a one-second rate, 15 of the digits are odd (or even), and the subject monitors the screen and presses the same button as quickly as he can every time an odd digit appears. Two age groups are included: (1) 18-25 years and (2) 60-75 years. For the five trials (90 sec. periods), the time from the beginning of one trial to the beginning of the next is either two minutes or one hour.

Experiment XII is another study of visual information processing, stemming directly from the results of Experiment X. In Experiment X, several subjects responded sequentially (by visual fields rather than by simultaneous pairs) indicating sequential processing of visual information. Previous research in this laboratory showed that many (but by no means all) subjects typically responded sequentially in a dichotic-listening task. Experiment XII was designed to determine whether there are people who typically respond sequentially regardless of sensory modality. In other words, do some people process information sequentially for both visual and auditory inputs and others simultaneously for both modalities? In addition to digits visually presented with a tachistoscope, auditory digits are presented dichotically (one of each simultaneous pair to each ear). In both modalities, each presentation consists of four different digits: two simultaneous digits followed by two more simultaneous digits. The sample will consist of 32 men and women between 18 and 30 years of age.

Major Findings: In Experiment VII, the longitudinal study of memory for designs (Benton), relationships were found between change in performance and several scales of the Guilford-Zimmerman Temperament Survey (GZTS) among 52 men initially over seventy. This was the age group which previous analyses had shown to have substantial mean declines in Benton performance over a six-year period. About one fourth of the men over seventy, however, did not decline. As part of an attempt to characterize differences between men who declined and men who maintained their performance, residual change in Benton was correlated with the GZTS. Personality characteristics which predicted subsequent decline included: being less active, energetic, and productive (General Activity); being less responsible, serious, self-restrained, and more impulsive (Restraint); and being less analytic and task oriented (Masculinity). The multiple correlation between levels of these three scales and magnitude of change in Benton performance was .59. Changes in GZTS scores, however, were not related to changes in Benton performance. Levels of GZTS scores repeated six years after the initial measures were also related to magnitude of change in Benton performance for this group of men. Again the three scales (General Activity, Restraint, Masculinity) which predicted change in performance, when repeated, were correlated with that change. In addition, the repeated measure of Emotional Stability was correlated with change in Benton performance; men who were high in Emotional Stability on the second measure tended to decline less.

Preliminary cross-sectional analyses of initial Benton measures for the women indicated age differences similar to those found for the men, i.e., small differences early and large differences late in life. The correlation between age and errors was .49 -- quite similar to the cross-sectional correlations of .47, .47, and .51 previously reported for three samples of men in the BLSA. The levels of performance for the women were comparable to the initial measures of the men tested in the early 1970s.

In Experiment IX, the longitudinal study of the GZTS, stability was quite high. The six-year correlations for the total sample ranged between .71 and .83 with an average (using _z_ transformations) of .77 for the ten scales. Similar results were found for each of the three age groups (ranges: young = .68 - .85, middle = .69 - .86, old = .68 - .83; average correlations: young = .76, middle = .77, old = .75). The twelve-year correlations were also quite high. The range for the total sample was .68 - .83, and the average was .73. Ranges for the three age groups were: .63 - .85, .64 - .85, and .59 - .87. Average correlations were .72, .75, and .73. These correlations compare favorably with short-term correlations in other studies, and are among the highest long-term correlations in the personality literature. These results provide no evidence in support of the hypothesis that stability increases with age during the adult years. It should be pointed out, however, that the correlations for the young groups were so high (near the retest reliabilities) that there was little opportunity for the correlations of the older groups to be much higher.

Experiment X is the study of information processing using a visual analog of the dichotic-listening procedure. Pilot data had indicated that when each pair of digits was exposed for 75 msc (with no inter-stimulus interval), some young adults reported sequential pairs, i.e., the two digits presented in one visual field followed by the two digits presented in the other visual field. In a systematic study of 24 young adults, two exposure periods (75 and 125 msc.) were used with each subject and the two orders of exposure periods were counterbalanced. When the 75 msc exposure period was administered first, 5 of the 12 participants responded sequentially, i.e., predominantly by visual fields (rather than by simultaneous pairs); and 3 of those 5 continued to respond sequentially when the exposure was increased to 125 msc. When the 125 msc. exposure was administered first, 3 of the 12 participants responded sequentially; and all 3 continued to respond sequentially when the exposure was decreased to 75 msc. These results indicate that, for young adults, several (8 of 24) responded sequentially to digits when pairs were displayed for 75 msc., and 6 of these 8 responded sequentially when pairs were displayed for 125 msc. Contrary to statements in the literature, a substantial minority of young adults apparently process simultaneous, visual, verbal information sequentially rather than simultaneously.

Experiment XI is the response-time study of massed and distributed practice. Preliminary analyses indicate that neither simple reaction time nor choice reaction time consistently improved during the five trials in either the massed-practice or the distributed-practice conditions. After all the data are collected and analyzed, decisions will be made about whether further trials are necessary for learning to occur and/or whether other tasks should be explored to determine whether the elderly benefit from massed practice (as hypothesized from earlier animal studies in this Laboratory).

Experiment XII is a study of both visual and auditory information processing. Procedures were developed and data collection was initiated.

Significance to Bio-Medical Research and the Program of the Institute: The general idea that a person becomes more susceptible to interference as he grows older is well entrenched in gerontological thinking and is often used to "explain" age differences in performance. The evidence for this idea, however, is sparse and not consistent. It is the purpose of this project to explore the generality of the age-interference hypothesis for non-verbal memory and perception. It is important, both for theoretical and applied reasons, to identify those conditions which are especially interfering for the old. In addition, response slowing is a general behavior which pervades many aspects of information processing; another purpose of this project is to improve our understanding of information processing and how it changes with age.

Proposed Course of Project: Now that it has been established that some young adults respond sequentially to visually presented stimuli, indicating sequential rather than simultaneous information processing, old adults will be studied to determine whether they, too, show evidence of sequential processing of visual information. A related question is whether different exposure periods are optimal for eliciting sequential responding from old individuals. In the longitudinal study of memory for designs (Benton), personality (GZTS) predictors of change in memory performance will be analyzed for the entire age range of men, and other potential predictors will be explored.

Publication:

Robertson-Tchabo, E. A., Arenberg, D., & Costa, P. T., Jr. Temperamental predictors of longitudinal change in performance on the Benton Revised Visual Retention Test among seventy year old men: an exploratory study. In Hoffmeister & Müller (Eds.), Brain Function in Old Age, Proceedings of Bayer Symposium VII. Springer-Verlag, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00067-12 LBS

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Learned Modification of Visceral Function in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	K. Gaarder	Research Physician	LBS, GRC, NIA
	M. Garwood	Staff Fellow	LBS, GRC, NIA
	M. Glasgow	IPA Fellow	LBS, GRC, NIA
	A. Perski	Visiting Fellow	LBS, GRC, NIA
	E. Lakatta	Chief, Cardiovascular Section	CPB, GRC, NIA
	S. Gottlieb	Cardiologist	Balto. City Hospital
	W. Whitehead	Guest Worker	LBS, GRC, NIA
	M. Schuster	Gastroenterologist	Balto. City Hospital
	S. P. Tzankoff	Senior Staff Fellow	CPB, GRC, NIA

COOPERATING UNITS (if any)

Columbia Medical Plan
Baltimore City Hospitals
The Johns Hopkins University School of Medicine

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Psychophysiology

INSTITUTE AND LOCATION

GRC, NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 6.35	PROFESSIONAL: 4.85	OTHER: 1.50

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the interaction of behavior and physiology in man. One study investigates behavioral procedures (relaxation and biofeedback) in the control of blood pressure in patients with high blood pressure; a second study investigates heart rate biofeedback in patients with angina pectoris; another study investigates the physiological mechanisms of heart rate control during exercise in normal man; one study investigates bowel function in normal man and patients with irritable bowel syndrome; another study is designed to learn whether patients with histories of meningomyelocele and fecal incontinence can be trained to become continent; one study investigates age differences in autonomic response patterns; one study investigates age differences in electrodermal activity.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: A. To evaluate the clinical effectiveness of relaxation and biofeedback in the control of blood pressure in hypertensive patients.

B. To determine if patients with angina pectoris can learn to slow their heart rates, and can transfer this skill to an exercise stress test.

C. To identify the mechanisms underlying learned, voluntary control of heart rate during exercise in normal man.

D. To determine whether patients with histories of meningomyelocele and fecal incontinence can be trained to become continent.

E. To identify the behavioral and physiological criteria which characterize patients with irritable bowel syndrome, and to develop diagnostic criteria for differentiating sub-types of patients with irritable bowel syndrome.

F. To describe age differences in individual patterns of autonomic responses.

G. To identify the mechanisms underlying age differences in electrodermal activity.

Methods Employed: A. Patients were selected from the outpatient service of the Columbia Medical Plan. All patients have been diagnosed as suffering from high blood pressure. Study patients are limited to those who are receiving no antihypertensive medication and who have clinic diastolic pressures ≥ 90 mm Hg, and those patients who are receiving diuretic therapy (only) for their hypertension regardless of their clinic pressures. One hundred fifty-eight patients have participated in a multiphasic, controlled study. Phase 1 is one month long and is a baseline phase. All patients record their blood pressures 9 times/day (3 times upon awakening; 3 times during the middle of the day; three times during the evening). In addition, patients have their pressures taken approximately once weekly by a health professional. In phase 2 patients are assigned to one of three groups: self administered relaxation, self administered blood pressure biofeedback, control. Assignment to groups in phase 2 is such that the three groups are matched in blood pressures, and such that approximately equal numbers of patients in each group are either untreated or are taking diuretics. Phase 2 is 3 months long. Phase 3 is similar to phase 2 in terms of duration and nature of the treatments: control patients continue as controls; relaxation patients are subdivided into two groups--group A continues to relax, group B is trained in the feedback condition; and feedback patients from phase 2 are divided into two groups similarly to the relaxation patients. In phase 4, control patients receive six months of therapy (relaxation followed by feedback); patients on diuretic medication have their diuretics withdrawn if their diastolic pressures F 86 mm Hg, and they are followed as before; patients who have received only one therapy (relaxation or feedback) are offered the other treatment; patients who have received both therapeis continue to be followed for 6 months. All patients in phases 2-4 continue to record their pressures daily and also have their pressures taken weekly by a health professional. It is expected that all of the subjects will have finished phase 3 before the end of 1979 but that a few subjects will be in the follow-up stages for several more months.

B. A group of about 24 male patients experiencing angina pectoris in association with medically verified coronary artery disease will be studied. All patients will be referred from the outpatient service of Baltimore City Hospitals. Each patient will be given an exercise stress test to determine the level of heart rate (HR), systolic blood pressure (SBP) and the product of HR and SBP, the rate-pressure product (RPP), at which he experiences pain. Then he will be trained during 25 sessions to attenuate his HR while exercising on a bicycle ergometer. Following such training he will again receive an exercise stress test to determine whether the training in the laboratory improved his performance on the stress test. Half of the group will be control patients and will exercise on the bicycle without feedback training. They also will receive pre- and post-exercise stress tests. Finally, the control patients will receive training in HR control as did the experimental patients. In addition, followup procedures will enable the assessment of training on daily routines.

C. Two studies were done with normal, healthy subjects to assess the ability of these subjects to attenuate heart rate (HR) while exercising. In study I, ten, young, physically untrained subjects exercised on a bicycle ergometer at about 50% of maximal HR. Five subjects were in the experimental group and five were in a control group. The experimental subjects received feedback to slow HR while exercising during five sessions, the control subjects merely exercised for five sessions. Following training, the experimental subjects exercised for two additional sessions without feedback to determine whether they could transfer the skill that they had learned; the control subjects were trained for five additional sessions with feedback. In study II, ten, young, physically conditioned subjects (trained athletes) were studied in an experiment which was similar to Study I, but with the following additions: 1) All subjects received maximal exercise stress tests before and after training--the experimental subjects received two stress tests, one before training and one after training; the control subjects received three stress tests, one before entering the control exercise period, one after the control period, and the third after HR training; 2) During the stress tests serum levels of epinephrine, nor-epinephrine and blood lactate were measured, and oxygen consumption was measured; 3) Catecholamine levels in the serum and lactate levels as well as oxygen consumption were measured during specified experimental sessions.

D. Eight patients with lifelong histories of fecal incontinence secondary to meningomyelocele were studied. All but two children were maintained on enemas or suppositories prior to treatment, and all were unable to have appropriate bowel movements.

Diagnostic evaluation and biofeedback training were accomplished using a rectal tube as follows: The patient was placed in the left lateral position and a hollow cylinder 1.2 cm in diameter to which two doughnut-shaped balloons were tied was inserted into the anal canal so that one balloon was surrounded by the external anal sphincter. A third balloon 5 cm in length was inserted through the cylinder into the rectum and was used to distend the rectum. Momentary rectal distensions from 5 ml to 60 ml of air in increments of 5-10 ml were injected and immediately withdrawn in the rectal balloon.

Training consisted of 4 phases. Phase 1 was a diagnostic procedure which assessed the ability of the patient to contract the external anal sphincter prior to training and the minimum volume of rectal distension which the patient could perceive. In Phase 2 the patient was taught to emit an appropriate contraction of the external anal sphincter in the absence of rectal distension by providing visual feedback of the amplitude of his response and verbal praise. In Phase 3 the patient was instructed to contract the external sphincter whenever he perceived distension of the rectum by balloon. Beginning with the lowest level of rectal distension which the subject could perceive the volume of rectal distension was gradually increased as the subject acquired an ability to overcome reflex inhibition of external sphincter tone by voluntarily contracting the external sphincter. Phase 4 was a continuation of Phase 3 but without visual feedback. Verbal praise for appropriate responses also was gradually withdrawn.

Each training session was 60-90 min long and consisted of approximately 40 training trials. Training sessions were scheduled at two-week intervals for a maximum of 6 sessions. Patients were asked to record occurrences of incontinent episodes, appropriate toiletings, and assisted evacuation procedures for one week prior to training, throughout training, and for 4 weeks after training.

E. Twenty-eight consecutive referrals to the Digestive Diseases Division of Baltimore City Hospital who satisfied the diagnostic criteria for irritable bowel syndrome (IBS) were compared to 29 normal, control subjects. Inclusion criteria for the patient group were: 1) abdominal pain which was not relieved by eating or which was exacerbated by eating; 2) altered bowel habits (either constipation or diarrhea); and 3) absence of any diagnosable organic disease. When 3 patients were excluded who had uninterpretable motility studies due to technical error, there remained 5 patients who had diarrhea only, 15 who had constipation only, and 5 who had mixed symptoms (alternating constipation and diarrhea).

Colonic motility was investigated by positioning 2 ballons each 5 cm long so that the cephalad balloon lay at the junction of the sigmoid colon to the rectum and the second balloon lay in the rectum. These balloons were attached to polyethelene tubing which connected them to pressure transducers. Each balloon was inflated initially with 10 ml of air to permit recording of pressure changes.

The rectosigmoid ballon was distended in a stepwise fashion by injecting an additional 20 ml of air every two minutes up to a total of 180 ml. Contractions of the rectosigmoid colon and rectum were measured as monophasic pressure waves at least 1 mm Hg in amplitude. A motility index was computed by multiplying the amplitude of each delayed active contraction by its duration in seconds, adding these products for each two-min measurement interval, and dividing the sum by 4.

Seventeen of the 25 patients completed the Hopkins Symptom Checklist of psychophathology.

In order to rate the severity of the patient's symptoms, clinical histories were edited to eliminate any reference to colonic motility and were then blindly rated by a gastroenterologist not otherwise involved in the study. He assigned a score of 1 to 10 to each patient based on his overall impression of how troublesome and disruptive the patient reported his symptoms to be.

F. Test of the hypothesis that the ANS of the aged is functioning differently than that of young adults necessitates age comparisons on the interrelationships among ANS response measures. Comparison of age groups on the correlations between ANS response measures is inadequate because the ANS of young adults does not show a uniform response, and correlations among ANS response measures across individuals often are non-significant. The pattern of intra-individual ANS response thus needs to be investigated and the concept of individual specificity is a means by which to do this. Individual specificity refers to the tendency of an individual to emit the same hierarchy of responses to all stimuli. For example, hypertensives have been reported to have a greater tendency than normotensives to respond to all stimuli with a pressor response. Comparisons of age groups on individual specificity reflects age differences in the adequacy of ANS function because if an individual's psychophysiological response pattern is consistent across stimuli, his response pattern often will be inappropriate to the demands of the situation. Ten young subjects in each decade from 20 to 80 years are being tested. Each subject receives the following stimuli which are present in counterbalanced order for 30 seconds: (1) cold pressor, (2) mental arithmetic, (3) time estimation, (4) incongruous slides, and (5) exercise. These stimuli were selected because they are effective (i.e., elicit responses) and diverse, the appropriate stimuli by which to assess specificity. Subject responses are breathing rate, skin potential, digital blood flow, heart rate, and blood pressure. Intra-class correlations are used to assess individual specificity. Since health status data are collected as a part of the Baltimore Longitudinal Study of Aging, it will be possible to determine if individual specificity is related to disease and/or is predictive of disease.

G. Measurements of the electrical activity of the skin can provide information on age changes in skin function. For example, previous research in this laboratory has shown that there are age differences in one type of electrical activity--skin potential level (SPL). Old men have a less negative SPL than young men and this age difference appears to be due to a less negative sweat gland potential in old age. However, a number of physiological components contribute to the electrical activity of the skin. There may be age differences in other peripheral skin components as well as the sweat gland potential; these individual components of the electrical activity of the skin must be quantified. This research has focussed on developing a technique that permits quantification of these separate components.

Edelberg's model, the most widely accepted model of the electrical activity of the skin is used. His model specifies that the skin is comprised of two generators, a large voltage source in the sweat gland, and a smaller generator in the epidermis, and two resistors, the sweat duct and the epidermis. The sweat gland resistor and battery and the epidermal resistor and battery are each in parallel with the recording amplifier. Recorded voltage (SPL) is a composite of these components, and the relative resistances of the sweat gland and epidermal pathways determine whether recorded voltage primarily reflects the sweat gland or epidermal generators. Simultaneous measurement of SPL and skin resistance level (SRL) while varying epidermal hydration permits the quantification of the peripheral physiological components. Four recording channels are used: one channel of SRL and one channel of SPL are recorded with .5% KCl in 90% polyethylene glycol; one channel of SPL and one channel of SRL are recorded with .5% aqueous KCl. Previous research has shown that when SPL is more negative than resting levels, a more negative SPL is recorded with a glycol medium than with a hydrating medium. This difference in SPL negativity is due to hydration induced alteration in the ratio of epidermal resistance to sweat gland resistance. At rest hydration has no effect on SPL magnitude indicating that the sweat duct provides no effective current path and SRL and SPL recorded at this point reflect epidermal events. The subject is required to relax until similar SPLs are recorded with the two hydration conditions. At this point measurements are taken to determine the epidermal resistance and epidermal potential on each hydration channel. The subject then is activated by procedures such as mental arthmetic to produce a drop in SRL. SRL at this point and the previously obtained epidermal resistance measurements are used to derive the sweat duct resistance for each hydration condition. Measurements are made at the termination of the skin resistance response and attempts are made to elicit only uniphasic negative skin potential responses. This is done to avoid measurement contamination due to rapid changes in epidermal resistance. It is possible to use the previously obtained epidermal resistance measurement to derive the sweat duct resistance because the change in corneum resistance that takes place with sweat gland activity is a relatively slow process. By assuming that the potential across the sweat duct is the same during activation for the two hydration conditions, a predicted relationship between the two activated SPL channels can be obtained; the predicted values are compared to the obtained values. This general procedure of relaxation and activation to obtain values of epidermal and sweat duct resistance is carried out repeatedly to obtain multiple measurement points on individual subjects.

Major Findings: A. One hundred thirty-two patients have completed phase 1. Fifty-seven have completed the study and 40 are now in phases 2, 3 or 4, the remaining 35 either dropped out or were excluded for various reasons. It is too soon to report the treatment results except to note that almost all patients tested to date are able to show acute drops in blood pressure in conjunction with the relaxation or feedback procedures, while many treated patients have shown substantial and sustained drops of blood pressure into the normal range and a number of medicated patients have remained normotensive after discontinuing their diuretics. The baseline blood pressure data are now being analyzed. All of the subjects who have finished phase 1 (the baseline phase) of the study will be included. Currently, the analysis has been completed on 67 subjects and the data of the rest are being prepared for analysis. Preliminary findings in the analyses of baseline data include the following:

1) There are correlations between systolic blood pressure and age found with all of the measures of blood pressure (e.g., $r=.44$; $p<.001$ for average baseline period). These correlations are also found between blood pressure lability and age ($r=.23$; $p<.03$ for average of baseline period) and between the extent of drop of blood pressure during baseline and age ($r=.25$; $p<.02$ for average of baseline period). However, there are no significant correlations between diastolic blood pressure and age.

2) The health professionals' casual clinical readings, upon which blood pressure treatment is based, are frequently as much as 10-20 mm Hg higher or lower than the internally consistent home reading taken by the patient.

3) Blood pressure readings drop by about 7 mm Hg systolic and 5 mm Hg diastolic during the baseline period, with the drop most marked in the first week. This necessitates a minimum of about 4 weeks of baseline to assure the presence of stable, pre-treatment levels.

4) Afternoon systolic blood pressures are clearly about 5 mm Hg higher than morning or evening readings in group mean data. Morning systolic and evening diastolic readings are the lowest. This is contrary to the frequent assertion that blood pressure monotonically rises from morning through the evening.

5) Individual cases do not necessarily match the group mean. In individuals, highest and lowest systolic or diastolic pressures can be found at any of the three times of day.

6) Blood pressure patterns are based on the combinations of high, intermediate and low systolic and diastolic readings at the three times of day measured. Blood pressure variability adds to the complexity of the above patterns. These patterns may be related to the diurnal cycle of bioactive metabolites or to behavioral events varying according to the time of day. For example, if the blood pressure is consistently highest in the afternoon, this could be related to work, while highest pressures in the morning could be tied to poor sleep.

Some of the implications of this study for the treatment of hypertension in the elderly are: that over treatment due to over-estimation of blood pressure is less likely if home-recorded pressures are obtained based upon our comprehensive protocol; that a differential taxonomy of hypertension based upon patterns at times of day could detect age-related changes in etiology; that age-related behavioral liabilities such as poor sleep are more likely to be recognized.

B. Two experimental patients have completed the study and two control patients have completed the control phase of the study . Two patients are still active in the study, and several other patients are on a waiting list. Because the study is in its earliest stages, no meaningful analyses of the findings are possible. However, one preliminary finding is noteworthy: Both experimental patients showed an enhanced ability to reduce HR and RPP upon termination of the post-training, exercise stress test; neither control patient showed this recovery pattern.

C. In association with the feedback training the experimental subjects in Study I were able to significantly attenuate the exercise-induced increase in HR by about 20% relative to the control subjects. This same effect was seen in the control subjects when they underwent feedback training. Neither group showed a significant alteration in systolic blood pressure (SBP) relative to the control condition. Thus, feedback training led to a specific effect on HR with no effect on SBP. Consequently, the product of HR and SBP, rate pressure product (RPP), which is an index of left ventricular work, also was significantly attenuated during training. Finally, the experimental subjects participated in two, additional post-training sessions without feedback during which they showed that they were able to maintain the skill that they had learned, i.e., they continued to attenuate their HRs with no change in SBP.

Study II has just been completed and data analyses are beginning. A preliminary analysis of the data suggests that even physically conditioned athletes can attenuate the exercise-induced tachycardia during feedback training. This effect was present for the experimental subjects and for the control subjects during feedback training as well. SBP was similar in the control and feedback conditions so that RPP followed HR and also was attenuated. The data also suggest that oxygen consumption was attenuated during feedback relative to the control condition. Inspection of the results of the exercise stress tests indicate that the skill learned during training transferred to this test as well: HR and RPP but not SBP was lower on the stress test following training than it was on the stress test preceding training for both groups. Furthermore, oxygen consumption on the post-training stress test was lower than it was prior to training. Catecholamine responses, blood lactate levels and ventilation have not yet been analyzed.

D. Phase 1 testing indicated that the pathophysiology of incontinence in children with meningomyelocele differs in two ways from the pathophysiology of incontinence in the majority of patients with adult-onset fecal incontinence: 1) In children with meningomyelocele rectal distension produces active inhibition of external anal sphincter tone whereas in adult-onset fecal incontinence there is simply no response or a weak contraction of the sphincter following rectal distension; 2) The ability to sense rectal distension is normal in patients with meningomyelocele whereas sensation often is impaired in patients with adult onset fecal incontinence. Five of 8 patients in this study became completely continent as a result of less than 6 hours of biofeedback training, and a sixth patient decreased the frequency of incontinent episodes by 80%. These patients all maintained their treatment gains at follow-up 1.5-12 months after termination, and 5 of 6 discontinued all enemas or suppositories. Two additional patients were unable to produce any external anal sphincter contractions even without rectal distension; these two treatment failures were still incontinent at follow-up and still required frequent evacuation procedures. The clinical outcomes achieved in this study are superior to those produced by an enema or suppository regimen. The enema regimen produces continence in about 59% of meningomyelocele patients, but most patients must continue to take enemas or suppositories frequently as a prophylactic measure. Six of 8 patients in our study were incontinent although they were already on enema or suppository regimens prior to training. Two had not tried such a regimen. Moreover, the patients who completed the training program were able to discontinue enema or suppository regimens and to evacuate normally.

E. When the rectosigmoid colon is distended by adding 20 ml of air to the rectosigmoid balloon there occurs an immediate active contraction of the rectosigmoid colon having a peak within 10 sec of the air injection. Following the immediate active contraction, in IBS patients there frequently follow one or more delayed active contractions with pressure peaks occurring more than 10 sec after the air injection. The amount of motility in the rectosigmoid colon differentiates patient groups from each other and from normal subjects, but only when the rectosigmoid colon is stimulated. Prior to distension (i.e., under baseline conditions), neither IBS patients nor normal subjects exhibit spontaneous contractions of the rectosigmoid colon. However, when the colon is distended with progressively larger volumes of air, the motility index is found to be significantly ($p<.05$) greater in patients with diarrhea than in constipated patients at 20, 100, 120, 140, and 160 ml distension; and significantly greater in constipated patients than in normals at 60, 140, 160, and 180 ml distension. Patients with mixed symptoms fall midway between constipated patients and those with diarrhea. There are two recognizable types of delayed active contractions which appear to serve different physiological functions. Slow contractions of the colon (those lasting 15 sec or longer) are significantly more frequent in IBS patients than in normal subjects, but there are no differences in frequency of slow contractions between constipated patients and those with diarrhea. Fast contractions (less than 15 sec duration), on the other hand, are significantly more frequent in patients with diarrhea than in patients with constipation, and there are no differences between constipated patients and normals on this measure. In addition, slow contractions occur singly or at irregular intervals whereas fast contractions usually occur in runs at a fixed frequency between 6-9 cpm, the frequency varying slightly between individuals. The amount of colonic motility (motility index) was significantly correlated with blind ratings of the severity of clinical symptoms in diarrhea patients ($r=.90$) but not in consitipated patients ($r=.22$). In the Hopkins Symptom Checklist IBS patients received significantly elevated scores on global psychopathology scales and on the following clinical subscales, somatization of affect, interpersonal sensitivity, depression, anxiety, and hostility. Patients with diarrhea were not significantly different from constipated patients on any scale of the Hopkin's Symptom Checklist.

F. Data collection has been completed for the first test. Results are now being analyzed.

G. Results indicate that Edelberg's model has considerable predictive power. Spearman ranks correlations between predicted and obtained SPL among 8 subjects ranged from.90 to .99 and no intercept was significantly different from zero. Thus, it now may be possible to measure sweat gland potential itself.

Proposed Course of Project: A. This project will continue until 90 or more patients complete all phases of the study, which will occur during FY 80.

B. This project will continue until 24 patients have been fully tested.

C. Study I has been completed and is now in press. Data from Study II will be completely analyzed. Future experiments in this project will be designed to determine in greater detail the physiological and behavioral mechanisms underlying what now appears to be an important breakthrough in the study of exercise physiology.

D. A larger series of 40 incontinent patients with meningomyelocele will be studied, and additional physiological parameters will be monitored to determine the mechanism by which these patients learn bowel control. Additional measures will include sphincter EMG, and duration of the patient's ability to hold a water enema. Additionally, we will compare the efficacy of biofeedback based on the EMG signal to the manometric biofeedback used previously.

E. Future studies will examine in more detail the psychological characteristics of IBS patients. In particular we will examine the hypothesis that excess motility of the colon leading to symptoms of IBS is due in part to faulty learning which may occur when parents and others in the environment respond positively to and thereby reinforce the occurrence of symptoms. This research aims to explicate the psychophysiology of IBS with the objective of developing more effective behavioral medicine treatments for this disorder.

F. Data collection will continue. Longitudinal studies, i. e., repeat testing of the subjects, is projected at two years and ten years.

G. The technique will now be used on older subjects to determine age differences in peripheral skin characteristics. It will also be used on certain patient groups with known alterations in skin function such as patients with cystic fibrosis of the pancreas to further validate the technique.

Significance to Biomedical Research and the Program of the Institute:
A. Hypertension research has been hindered by the lack of a reliable measure of blood pressure and of a method of adequately determining baseline blood pressure. This program makes a significant contribution toward both of these goals by providing reliable measurements which can be used as a baseline. This is especially important in the evaluation of hypertension in the elderly, where a strong iatrogenic effect can lead to differences between office blood

pressure readings and home blood pressure readings which can lead to faulty diagnosis or treatment. The behavioral treatment of hypertension is a current topic of great interest in the medical community and a well-done study will settle many questions. A behavioral treatment is especially relevant to the elderly because they respond less reliably to medication, and because systolic hypertension is apt to be aggravated by increases in cardiac output which may be especially amenable to behavioral treatment.

B. Angina pectoris is a serious disease whose likelihood increases with advancing age. Furthermore, it is a disease which greatly limits the quality of life. Any technique which can improve the ability of such patients to perform in their daily lives will have considerable value.

C. Cardiovascular adjustments to physical conditioning are attributable to processes mediated through the nervous system as well as mechanical adjustments which take place in the heart, blood vessels and in working muscles. Very little is known about the way in which the neurally-mediated effects occur or about the way in which these effects are determined by behavioral mechanisms. This research will be fundametal in understanding these processes.

D. Meningomyelocele effects 0.1% of births and approximately 40% of patients with meningomyelocele have fecal incontinence. Therefore, there is a large population of patients for whom these procedures have relevance. Additionally, we should be able to apply these procedures to adults with incontinence secondary to other types of CNS injury based upon our experience with these young patients.

E. Because IBS affects an estimated 8% of the adult population at any one time and accounts for approximately half of consultations to gastroenterologists, this research aimed at its more effective treatment has social relevance. By better understanding the physiology of the colon we may be better able to treat the major gastrointestinal disorder of old age which is diverticulosis. It has been suggested that diverticulosis may be the end result of the excessive colonic motility associated with IBS.

F. Diseases of the autonomic nervous system (e.g., hypertension, coronary artery disease) predominate in old age. The life history of the individual is likely involved in the development of these disease processes. Autonomic specificity is one aspect of an individual's life history--the development of an individual's autonomic nervous system response to different situations. In studying a person's pattern of autonomic nervous system response over time it may be possible to determine maladaptive reactions that are related to later disease development. For example, a life-time pattern of an overreactive pressor response may be involved in hypertension. The study of individual specificity enables these proposed relationships to be investigated.

G. Adequacy of function of the peripheral sudomotor system is crucial to older individuals. There is a reduction in sweat gland response which impairs the older person's ability to regulate temperature. Older people have drier skin than younger people which not only is a cosmetic problem but also may be related to a loss of the skin's protective function. This research program is involved in developing adequate measures of sudomotor function so that age-related alterations and their significance can be investigated in the human.

Publications:

Whitehead, W. E., Aleo, S., Edwards, T. L., Jr., Engel, B. T., Gregory, C., Latimier, P., and Schuster, M. M.: Biofeedback in the Treatment of Gastrointestinal Disorders: Task Force Report of Biofeedback Society of America. Biofeedback Self-Reg. 3: 375-384, 1978.

Baile, W. F., Engel, B. T.: A behavioral strategy for promoting treatment compliance following myocardial infarction. Psychosom. Med. 40: 413-419, 1978.

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Engel, B. T.: Behavioral applications in the treatment of patients with cardovascular disorders. In Basmajian, J. V. (Ed.) : Biofeedback-Principles and Practice for Clinicians. Baltimore, Williams and Wilkins, 1979, pp. 170-179.

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Whitehead, W. E., and Schuster, M. M. Functional gastrointestinal disorders. In Gallon, R. (Ed.): Treatment Approaches to Psychosomatic and Related Disorders, in press.

Schneiderman, N. S., Weiss, and Engel, B. T. Modification of psychosomatic behaviors. In Davidson, R. S. (Ed.): Modification of Pathological Behavior. New York, Gardner Press, 1979, pp. 233-272.

Garwood, M. K., Capriotti, R. M., and Engel, B. T. Psychosomatic aspects of disease in aging women. Female Patient, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00068-17 LBS

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Operant Performance, Memory and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles L. Goodrick	Research Psychologist	LBS GRC NIA
OTHERS:	None		

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Learning & Problem Solving

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS: .20	PROFESSIONAL: .10	OTHER: .10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major purpose of this project is to analyze complex maze learning of young and aged animals, and to determine techniques which act to improve learning ability. Another goal of this project is to determine age differences in operant performance for young and aged rats or mice, and to determine factors which may act to improve performance, and also improve retention of, the learned responses.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The objectives of one phase of this project are: (1) to study age differences in motor performance during operant responding; and (2) to develop operant techniques which improve the retention of learned responses in aged animals. The general objectives of the other phase of this project are: (1) to analyze complex maze learning of young and aged animals; and (2) to determine variables which may act to enhance or retard maze learning ability.

Methods Employed: Operant conditioning performance and retention studies have used 2-bar test boxes in which hungry animals are trained to press one bar to obtain a food reward while the alternate bar remains neutral. By increasing the complexity of the task (using two bars rather than one), it is possible to make a finer analysis of performance and the retention process. We are studying performance and retention as a function of reward schedule, and we are particularly interested in the partial reinforcement effect. The retention of partially rewarded responses is vastly greater than responses continuously rewarded; and analysis of this phenomenon will provide information regarding the general retention process.

A complex 14-unit multiple-T maze also is utilized. This maze has been shown to be a highly reliable test of learning, and it has been used in many major studies of aging. Additional mazes of 6-units are being developed to study mastery of consecutive problems by young and aged rats. These mazes will be used to analyze aging effects in short-term and long-term memory and to determine aging effects in interference, both proactive and retroactive.

Major Findings: None

Significance to Bio-Medical Research and the Program of the Institute:
Learning and/or memory deficits represent a major problem among the aged human population. Major behavioral techniques to reduce performance deficits obtained for aged animals have been studied in our laboratory. This project may facilitate research with man by identifying optimal conditions for learning and for retention of learned responses.

Proposed Course of Project: Further studies are in progress to determine the nature of the partial reinforcement effect in relation to: (a) time contingent vs. response contingent partial reinforcement; and (b) massed vs. distributed extinction trials. Other studies will examine age differences in operant performance as a function of response effortfulness. Maze studies will concentrate upon the effects of central nervous system stimulants on behavioral rigidity within the maze for old rats. We have also initiated preliminary studies of memory in aged rats to develop a model system to study neurohumoral correlates of memory.

Publication:
Goodrick, C. Problem solving and age: A critique of rodent research. In R. Sprott, (Ed.): Age and Intelligence. Van Nostrand Reinhold, New York, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00069-14 LBS

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Exercise, General Activity Level and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles L. Goodrick	Research Psychologist	LBS GRC NIA
OTHERS:	Teena M. Wax		

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Learning and Problem Solving

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD. 21224

TOTAL MANYEARS: .80	PROFESSIONAL: .40	OTHER: .40

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major goal of this project is to determine the effect of voluntary wheel exercise upon behavior for animals tested in lifespan developmental research, and in addition to consider the effect of voluntary wheel exercise upon longevity. Another goal of this research is to increase the period of vigorous activity during later stages in the lifespan of the rat or mouse.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The general objectives are: (1) to determine methods for increasing vigorous physical activity of lower animals during late stages in the life span; (2) to examine behavioral and longevity differences among animals which differ in physical activity level; and (3) to determine the physiological mechanisms underlying differences in activity.

Methods Employed: Wistar rats or various strains of mice are placed in standard activity wheels and allowed access to free voluntary exercise. Hungry animals also may be rewarded with food for running. A technique to experimentally control body weight and to increase voluntary exercise utilizes feeding of animals on a periodic schedule, such as every other day. Other studies utilize inbred, hybrid, and mutant mice or species which differ in activity level due to different genetic constitutions (See Project Z01 AG 00061-15 LBS Behavioral genetics and aging).

Major Findings:

A. Last year we reported a study of exercise and aging for male and female Wistar (GRC colony) rats. These rats were fed ad libitum with the major result an increase in life span for rats allowed exercise, compared with control rats not allowed exercise. In another study, 1.5 month old male rats were allowed food for 24 hours, then fasted for 24 hours with these dietary procedures continuing throughout the life span (periodic fasting, fed every other day = EOD); 24 were allowed exercise and 24 were in standard cages. Two other groups of male rats of the earlier study were fed ad libitum (AL); 40 were allowed exercise and 28 were in cages. In addition, another control group is now available (30 male rats in cages fed AL). Mortality curves of two AL fed male control groups (not overlapping in time) were completed during the nearly four year period of EOD testing.

Correlations of longevity and growth rate, longevity and growth duration, and longevity and peak weight were obtained for each group (see Table 1). All correlations of longevity and growth rate were negative and statistically significant; rats which increased in body weight slowly lived longer. Correlations of longevity and growth duration were all positive and statistically significant; rats which increased in body weight over a long time period had a long life span. The correlations of longevity and peak weight were positive and significant only for ad libitum fed rats; a high peak body weight was related to a long life span (the heaviest rat, 777 gm., of the second control group lived the longest of the 30 rats in this group).

Means and standard errors for longevity, growth rate, growth duration, and peak body weight are given in Table 2. The mean longevity of rats maintained on schedules of periodic fasting was significantly increased compared with controls fed ad libitum. Comparing rats fed ad libitum and maintained in cages with rats fed on a periodic fasting schedule, the increment in life span is 53% for group a and 89% for group b. Mean growth rate of rats periodically fasted was lower, growth duration longer, and peak body weight lower, compared with rats fed ad libitum. The amount of voluntary wheel exercise of rats fasted periodically also was maintained at a higher level late in the life span compared with the voluntary wheel exercise of ad libitum fed rats.

B. This experiment was designed to determine voluntary exercise of rats when exercise (wheel movement by walking or running) was the response which resulted in access to food pellets. Mature and aged rats were studied for 101 days to determine their ability to maintain body weight on different work requirements after periods of food restriction. Characteristic work patterns were obtained and analyzed over 24 hour periods for young and aged rats. After extensive training procedures, it was possible to determine age differences in motivation and age differences in patterns of work responses for food rewards under conditions in which work requirements resulted in equivalent body weight maintenance for the individuals of the two age groups. This information will be useful in studies of the prolongation of behavioral and physiological vigor late in the life span and in studies which require the maintenance of higher than normal levels of wheel exercise under minimally stressful conditions.

Control rats were placed in the cage-wheel units for 101 days with food and water ad libitum. The experimental groups were fed once a day (8 grams of rat feed) for the first 24 days. During this period each experimental rat received five 2-hour training sessions (days 16, 17, 18, 22, 23). During these periods the rats learned that movement within the wheel would activate the pellet feeder. The final two sessions required seven wheel revolutions (Fixed Ratio 7) for each reward food pellet. Following the five training sessions, all rats were placed on ad libitum feeding in the home cage-wheel unit and weekly 24-hr. work sessions were started. The work schedules are given in Table 3. Each week for seven weeks experimental rats were given a 5-day period of ad libitum food, then 24 hours of food deprivation, and a 24-hour work session. To obtain information with respect to age differences with increasing motivation, two sessions (8 and 9) had 48 hours of food deprivation. The last period of food deprivation was followed by 15 consecutive days (360 hrs.) of work during which all food was earned. For all work sessions the aged rats were required to do half the work required of young rats for equivalent amounts of food.

When food rewards were given to the young rats contingent upon wheel movement, the result was a consistently high level of activity compared with young controls living in cages with attached wheels. Significant differences were obtained between these groups for every 24-hour work session (experimental vs. control). It is of interest that during the initial days, wheel activity group differences were low, but on most nonwork days the experimental groups were higher in voluntary wheel exercise (days 24-95, on 41 out of 54 nonwork days activity was higher for young experimental rats than for young controls). Usually after a work session, a marked drop in voluntary wheel exercise occurred. During these nonwork days, the young control rats averaged about 2,000 revolutions per day while the young experimental rats averaged about 4,000 revolutions per day. Therefore, for the young the increment in activity from nonwork control sessions to work sessions was 400 to 500 percent, an increment from 2,000 to 8-10,000 wheel revolutions.

The control and experimental aged rats were similar in voluntary wheel exercise means on all nonwork days, normally less than 100 revolutions per day. However, on work days wheel activity increased greatly for aged experimental rats to a level 3,000 to 4,000 percent of normal, and the levels of wheel activity on the last days of work attained activity increments of the same magnitude as for the young experimental group.

On the initial 24-hour training session (FR7 and FR15) there was a mean body weight increment for both aged and young groups. When the work requirement was doubled (FR 15 and FR 30), a mean body weight decrement occurred for both young and aged groups on 3 of 4 work days. When the rats were continued on this work schedule at days 87-96, a continued reduction in weight resulted despite an increase over days in rewards obtained. When the work schedule was reduced (FR 12 and FR 24 on days 97-101), body weight stabilized.

Differences in patterns of activity (wheel exercise work) were obtained for young rats versus aged rats. These age differences in patterns of activity were not due to the differences in FR schedules. Motivation (24 hr. deprivation vs. 48 hr. food deprivation) also had no consistent effect on pattern of activity or total activity during work days; the major effect of food deprivation was a modest increment in activity on the second day of deprivation. Means are given in Table 4 for the last 3 of 10 consecutive work days (days 94-96) on FR 15 (aged) or FR 30 (young). Differences obtained were highly reliable and statistically significant and were consistent on every work day. The amount of wheel activity (revolutions) was significantly higher for young than aged rats ($\underline{F}$ (1,4) = 52.13, $\underline{p} < .01$) although the duration of activity was similar ($\underline{F}$ (1,4) = 2.20, N. S.). Compared with the young rats, the aged rats engaged in significantly more bouts of activity ($\underline{F}$ (1,4) = 14.58, $\underline{p} < .01$), had activity bouts of shorter duration ($\underline{F}$ (1,4) = 8.37, $\underline{p} < .01$) and were slower in speed of activity ($\underline{F}$ (1,4) = 29.48, $\underline{p} < .01$). Days and the interaction of days and age were not statistically significant in any of the above analyses. Although maximal wheel activity was significantly higher for young than aged rats, the increment in wheel activity (about 6,000 wheel revolutions) above base levels was similar for both young and aged rats. Thus, although physical ability differed, motivation was the same for young rats and rats of advanced old age.

Significance to Bio-Medical Research and the Program of the Institute: One of the consistent findings of gerontological research is the decline in general activity level of old animals compared with young animals. It is important to determine whether quantity of activity (e.g., wheel activity) and/or quality of activity (e.g., increased exploration behavior or greater response variability) may be increased experimentally for old and senescent animals. It is also important to examine the role of heredity with respect to voluntary exercise throughout the entire lifespan and the effect of exercise upon behavioral decrements associated with advanced old age. The knowledge and utilization of factors which change base activity levels of aged animals may result in the development of methods which can increase the productive later years of aged humans.

Proposed Course of Project: The rat studies of wheel exercise will continue to determine the effects of voluntary exercise upon longevity for paired rats and to determine the amount of voluntary exercise during avanced old age. Additional studies will determine the effect of food restriction (periodic fasting) upon voluntary wheel exercise and longevity for groups started late in the life span. Control groups will be added to obtain data with respect to normal longevity.

Studies of wheel exercise periodicity of young and aged mice will be continued. Periodicity patterns of mice will be examined throughout old age. Additional studies will determine the level of voluntary activity for young and aged mice which are allowed voluntary control of lighting conditions within the home environment. Studies of the effect of dietary protein changes late in the life span upon voluntary wheel exercise and longevity for rats will continue.

Publication:
Goodrick, C. Effects of long-term voluntary wheel exercise on male and female Wistar rats: Longevity, body weight, and metabolic rate. Gerontology, In press.

Table 1

Correlations of Longevity and Growth Rate, Longevity and Growth Duration, and Longevity and Peak Body Weight as a Function of Exercise vs. Non-exercise and Periodic Fasting vs. _ad libitum_ Feeding

Ad Libitum		Longevity X Growth Rate	Longevity X Growth Duration	Longevity X Peak Weight
Exercise^{c}	(N = 40)	$-.57^{*}$	$+ .47^{*}$	$+ .73^{*}$
Control^{c}	a(N = 28)	$-.43^{*}$	$+ .51^{*}$	$+ .39^{x}$
Control	b(N = 30)	$-.79^{*}$	$+ .93^{*}$	$+ .59^{*}$
Periodic Fasting				
Exercise	(N = 24)	$-.61^{*}$	$+ .74^{*}$	+ .34
Control	(N = 24)	$-.54^{*}$	$+ .63^{*}$	- .01

^{x} $\underline{p} < .05$

* $\underline{p} < .01$

^{c} Groups from a previous study.

Table 2

Means and Standard Errors for Longevity, Growth Rate, Growth Duration, and Peak Body Weight as a Function of Exercise vs. Non-exercise and Periodic Fasting vs. *ad libitum* Feeding

Ad Libitum	Longevity (weeks)	Growth Rate (gm/week)	Growth Duration (weeks)	Peak Body Weight
Exercise[c] (N = 40)	107.0 ± 3.0 (24.7 mo.)	8.2 ± 0.3	69.7 ± 1.7	555.5 ± 6.3
Cage[c] a (N = 24)	89.6 ± 2.6 (20.7 mo.)	11.8 ± 0.3	58.5 ± 1.7	677.3 ± 9.2
Cage b (N = 30)	72.9 ± 2.7 (16.8 mo.)	12.9 ± 0.6	52.6 ± 2.0	649.8 ± 14.2
Periodic Fasting				
Exercise (N = 24)	117.6 ± 4.9 (27.2 mo.)	6.2 ± 0.4	77.1 ± 3.6	453.7 ± 10.5
Cage (N = 24)	137.8 ± 4.1 (31.8 mo.)	4.3 ± 0.2	98.1 ± 3.4	406.5 ± 8.4

[c] Groups from a previous study.

Table 3

Food Regimen or Work Schedule for Young and Aged Rats on Days 24 Through 101.

Day	Young	Aged
24-28	AL	AL
29	FD	FD
30	FR 15[x]	FR 7[x]
31-35	AL	AL
36	FD	FD
37	FR 15[x]	FR 7[x]
38-42	AL	AL
43	FD	FD
44	FR 15[x]	FR 7[x]
45-49	AL	AL
50	FD	FD
51	FR 15[x]	FR 7[x]
52-56	AL	AL
57	FD	FD
58	FR 15[x]	FR 7[x]
59-63	AL	AL
64	FD	FD
65	FR 30[x]	FR 15[x]
66-70	AL	AL
71	FD	FD
72	FR 30[x]	FR 15[x]
73-77	AL	AL
78-79	FD	FD
80	FR 30[x]	FR 15[x]
81-84	AL	AL
85-86	FD	FD
87-96	FR 30[x]	FR 15[x]
97-101	FR 24[x]	FR 24[x]

[x] Work days (all other days are nonwork days).

(AL = Ad libitum food, FD = no food, FR 7 = 7 revolutions/pellet, etc.)

Table 4

Means and Standard Errors for Total Wheel Activity, Duration of Activity, Bouts of Activity, Duration of Activity Bouts, and Speed of Activity (Young = FR 30, Aged = FR 15).

	8 (day 94)	9 (day 95)	10 (Day 96)
	Mean Total Wheel Activity in Hundreds of Revolutions		
Young	103.0	104.0	99.3
Aged	51.7	54.3	54.3
	Mean Duration of Activity (Min.)		
Young	452.6	468.7	403.3
Aged	557.3	583.7	583.3
	Mean Bouts of Activity		
Young	10.0	10.7	8.0
Aged	19.7	18.7	22.3
	Mean Duration of Activity Bouts (min.)		
Young	47.5	44.7	50.3
Aged	28.2	31.4	26.4
	Speed of Activity (Rev./min)		
Young	23.0	22.2	24.4
Aged	9.7	9.6	9.5

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00075-01 LBS

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Stress, Coping and Personality in Aging Men and Women

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul T. Costa, Jr.	Chief, Section on Stress and Coping	LBS	GRC	NIA
Other:	Robert R. McCrae	Senior Staff Fellow	LBS	GRC	NIA
	Eleonore Lehr	Visiting Fellow	LBS	GRC	NIA
	Stanford Lamberg	Chief, Dermatology	Baltimore City Hospitals		

COOPERATING UNITS (if any)

Department of Dermatology, Baltimore City Hospitals
Section on Learning and Problem Solving

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Stress and Coping

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.25	2.5	2.75

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the effects of stresses, coping processes, and enduring personality dispositions on psychological and health outcomes. One study investigates the determinants of subjective well-being; a second examines the influence of daily moods and stress on psoriatic condition; a third measures personality and coping styles through interviews and projective tests; a fourth investigates the antecedents and expressions of the personality disposition of Openness to Experience; the fifth examines existing longitudinal data to determine age-related constancy or changes in personality and well-being; the sixth examines self-reported health as a function of personality, age and biomedical status; the seventh study investigates relations between individual differences in personality and cognitive performance over the adult life span.

Project Description

Objectives:

The program of the Section on Stress and Coping, LBS, is devoted to an investigation of the stresses faced by aging adults, the methods and strategies used by them to cope, and the effectiveness of their coping efforts. Stresses are viewed as events or difficulties which the individual encounters. They lead to attempts to cope, and the success of these coping efforts is judged by various measures of adaptational outcomes. Personality and life situations are seen as determinants of all three of these categories of variables. Personality is defined as the organization of mechanisms for adaptation or coping, and the three domains of personality in our model-Neuroticism, Extraversion and Openness to Experience-each have important implications for the process of coping. The development of life structures - careers, friendships, marriages - similarly represent global and vital forms of coping with inner needs and social demands. It is within this broad context of an aging personality in a complex social environment that the study of specific coping and adaptational mechanisms will be made.

Specific project objectives:

A. To evaluate the relative influence of age, personality, life events, objective circumstances, and adaptational processes on subjective well-being.

B. To determine prospectively whether emotional states or stressful events exacerbate the extent of psoriasis.

C. To measure personality and coping styles through a variety of non-self-report methods.

D. To examine parent-child relations as antecedents, and sentence completion responses as expressions of the personality disposition of Openness to Experience.

E. To describe maturational changes in personality, well-being, and psychosocial functioning in adults.

F. To identify the determinants of self-perceived health.

G. To explore relations between individual differences in personality and performance on cognitive tests and mental performance tasks, especially visual memory and other short-term memory tasks.

Methods Employed

A. Subjects were 903 adult male volunteers, part of an on-going longitudinal study (Normative Aging Study, Boston, MA). Over a period of nine months, four questionnaire packets were mailed to subjects. Measures of subjective well-being, personality and life events were included; specifically: the Bradburn Affect Balance Scale; the Beck Hopelessness Scale; the Knutsen Personal Security Inventory; a Life Satisfaction Index; the Buss & Plomin EASI-III Temperament Scales; The Eysenck Personality Inventory; scales from the Cattell 16PF; and the Holmes-Rahe Schedule of Life Events. Extension of this research will employ alternate measures of both personality and subjective well-being already collected or to be administered by mail to the men and women of the Baltimore Longitudinal Study of Aging and their spouses. In addition to recent life events, stress will also be measured as anticipated life events, and daily stresses and strains. The assessment of this range of stresses will allow an examination of adaptation levels. Specific measures to be collected include: The Profile of Mood States; Schedule of Recent and Anticipated Events; Daily Events checklist; a Well-Being Assessment; the Eysenck Personality Inventory, Form A; the NEO Inventory, and the Parent-child Relations Questionnaire.

B. A group of 30 psoriatic patients will be compared with three non-psoriatic control groups in terms of stressful events, personality, and mood. Control groups will be spouses, patient's siblings, and clinic controls who have skin diseases unrelated to stress. All subjects complete an initial battery of personality and well-being measures, and a history of recent life events. Patients, spouses and siblings will complete a second questionnaire at the end of 7 weeks which will include personality and measures of self-perceived health; patients and spouses will complete a re-test of first battery tests at the end of the three-month project to allow assessment of re-test stability or change. On alternate days, both patients and spouses will fill out check lists of moods, states, or daily events. Finally, the patients will be examined at Baltimore City Hospitals every two weeks in order to measure any changes in extent or severity of psoriasis. The design will permit retrospective and prospective analysis of the effect of stress on subsequent exacerbation of psoriasis, as well as an examination of the effects of psoriasis on subjective well-being and the possible mediating effects of personality and moods.

C. Subjects for this study will be participants in the Baltimore Longitudinal Studies of Aging (BLSA). In order to supplement and validate the self-report questionnaires used in the other studies, a number of other methods of assessment will be employed. Responses to open-ended questions dealing with stresses and wishes in the Activities and Attitudes Questionnaire collected over the past 20 years are being content coded prior to computer analysis. In addition, interviews and projective tests will be administered to BLSA participants on a six-year cycle, beginning this year with men, and including women next year. Interviews, which will be tape recorded for later re-scoring, intensively explore life stresses associated with family, friends, careers, and leisure activities. Inquiries also will be made concerning the patient's methods or strategies used to cope with stress. On another visit to the GRC, participants will be asked to take a battery of three perceptual-projective tests, including the Holtzman Inkblot Test, a psychometrically improved form of the Rorschach test; the Embedded Figures Test, a measure of the cognitive style of field independence, and three cards from the Thematic Apperception Test (TAT), including the University of Chicago's Intergenerational Card. A number of reliable coding systems exist by which to quantify data from the TAT. Data from all these sources will be analyzed in conjunction with objective data in classifying processes of coping, adaptation, and successful aging.

D. An objective self-report measure of Openness to Experience, the Experience Inventory, was administered to a large sample of adult male volunteers in an on-going longitudinal study (the Normative Aging Study, Boston, MA). From the responses of 800 men, aged 30-80, an item factor analysis indicated that 81 of the 84 items loaded on a general factor of Openness, thus providing strong evidence for the generality of the dimension of Openness. It was also possible to select items which formed six factorially discriminable subscales. Age trends in levels of Openness showed that rather than a steady decline, a saw-tooth pattern was obtained in which Openness was highest in cohorts born in 1920 and 1940 and lowest in cohorts born in 1930 and before 1910. This historical-cohort finding suggests hypotheses about the development of Openness which will be tested in the present research project by comparing retrospective childhood accounts of open and closed adults. This research will involve administration of the Openness measure as part of the NEO Inventory, a larger personality questionnaire. This will be incorporated in the mailing schedule planned for the BLSA participants and their spouses. The Parent-Child Questionnaire, asking subjects to retrospectively describe the relationship they as children had with their parents, also will be mailed to subjects. Joint analyses of these instruments will allow a retrospective assessment of the impact of certain child-rearing practices and attitudes in the formation of Open styles and dispositions. In another phase of this study, responses to Loevinger's Washington University Sentence Completion Test were obtained for 240 subjects and scored according to standard instructions for Total Protocol Rating and fluency. Additional personality measures were available on the same subjects.

E. Responses to the Guilford Zimmerman Temperament Survey (GZTS) have been collected since 1960 on male participants in the BLSA. Tests were re-administered at six-year intervals. Six-year re-test data were available for 460 men; twelve-year data for 222 men. In collaboration with Dr. David Arenberg, Chief, Section on Learning and Problem Solving, analyses of longitudinal stability and structure invariance were undertaken. Subjects were classified into three age groups (17-44, 45-59, and 60-85), and analyses were restricted to those who had taken their second GZTS 5.0 to 7.9 years after the first one and their third GZTS 11.0 to 15.4 years after the first. Reliabilities and true-score stabilities were estimated in addition to re-test correlation coefficients in the stability study. Structural invariance was examined by comparing factor analyses of three cohorts and three administrations of the GZTS. Coefficients of congruence were calculated to quantify comparison of factor structure. Administration of the GZTS will continue under the auspices of the Section on Stress and Coping. Other studies on the stability or change in personality and adaptation will involve analyses of the Activities and Attitudes Questionnaire, another measure on which data have been collected for a number of years.

F. A number of analyses have been conducted on self-perceived health in conjunction with personality and biomedical data. Self-perceptions of health are a key component of health maintenance since they influence efforts at self medication as well as the decision to seek medical treatment. Understanding the factors which determine self-perceived health is particularly important in dealing with aging persons who are likely to be facing new and increased medical-health problems. Self-perceived health is measured by responses to the Cornell Medical Index (CMI), a symptom checklist which includes both physical and psychiatric sections. The CMI has been administered to participants in the BLSA for a number of years. It is given in the first, fifth and ninth visits, and every fourth visit thereafter. Longitudinal analyses of sections of the CMI in conjunction with age and data from the GZTS were conducted for two and three administrations, taken from a sample of 1024 men. In addition, factor analyses were performed on the physical sections (A-L) of the CMI, with age and the Emotional Stability Scale of the GZTS as additional variables.

G. One of the best established changes with age is decline in certain forms of memory, learning, and problem solving. Speed of many mental operations, including decision making, also decreases. In one study, 124 male volunteer participants in the Normative Aging Study were given measures of personality and temperament including the Experience Inventory, Self-Control Scales, and the EASI-III Temperament Survey. Subjects also were measured on latencies in choice reaction, binary decision, memory for familiar and unfamiliar letters, and naming pictured objects. In a second study, conducted in collaboration with Drs. Arenberg and Robertson-Tchabo, longitudinal changes on the Benton Visual Retention test were correlated with personality dimensions in the GZTS. Subjects were 52 participants in the Baltimore Longitudinal Study of Aging who were over 70 at first administration of the Benton. Scales from the Guilford Zimmerman Temperament Survey were used in a multiple regression to predict changes in visual memory.

Major Findings:

A. All four measures of subjective well-being were significantly correlated, at a level suggesting substantial shared variance and construct validity. Correlations with personality scales from several instruments showed that measures of Neuroticism were negatively related to subjective well-being; while measures of Extraversion were positively related. A model of individual differences in happiness was presented which shows that one set of traits, (sociability, warmth, involvement with people, and activity), viewed as components of Extraversion, influences positive affect or satisfaction; while a different set of traits (anxiety, hostility, impulsivity and pyschosomatic concerns), seen as components of Neuroticism, influences negative affect or dissatisfaction. These two components are subjectively "balanced" by the individual to arrive at a net sense of subjective well-being, which may be measured as morale, life satisfaction, or simply happiness. The model stresses the independence of positive affect and negative affect in influencing subjective well-being, it thus calls attention to the separability of satisfaction from dissatisfaction, a phenomenon somewhat foreign to commonsense notions of happiness. So enduring are the effects of these two personality dimensions that well-being scores were predicted from personality measures collected ten years previously. In regression analyses in two age groups (35-59 and 60-85), personality measures were significant predictors of subjective well-being, while life events in the past ten years, including retirement, death of a loved-one, divorce or separation, and health events, were not. This finding of small association between objective circumstances and subjective well-being, was interpreted as evidence of adaptational processes.

B. Since data collection has just begun there are no findings to date.

C. Since the data collection has just begun there are no findings to date.

D. Correlations between objective personality measures and sentence completion scores show that several facets of Openness to Experience are positively related to level of ego development. By contrast, ego level is unrelated to measures of Neuroticism or Extraversion. Fluency, the number of words used by the subject in completing sentences, was unrelated to any objectively measured personality dimension. This finding shows that Open individuals are less likely to have conventional or conformist values, more complex thought, and more differentiated feelings. When the Loevinger test is construed in developmental theory terms, these data suggest that Open individuals have attained a higher level of maturity, perhaps because of childhood experiences.

E. Analyses of the GZTS over six- and twelve-year intervals on 406 male participants in the BLSA show evidence of predominant stability in personality, with re-test coefficients ranging from .59 to .87. Levels of stability appeared to be equally high in all age groups, from 17 to 85; and, when corrected for unreliability, traits in the domain of Neuroticism were observed to be as stable as those in the domain of

Extraversion. With regard to structure, three factors were found in each age group, corresponding to Neuroticism, Extraversion and Thoughtfulness-Restraint. Coefficients of congruence between solutions in different age groups ranged from .95 to .99, indicating structural invariance in the GZTS for middle-aged and older males. These conclusions are consistent with recent reviews of the literature on personality stability in adulthood.

F. Six- and twelve-year longitudinal analyses of the physical health sections (A-L) of the CMI in a sample of 1024 male participants in the BLSA aged 17 to 97 showed differential effects. With increasing age, increases were observed in sensory, cardiovascular and genitourinary problems or complaints. Neuroticism, on the other hand, showed relatively more diffuse effects. More neurotic subjects, (measured by the psychiatric sections of the CMI, (M-R) and the Emotional Stability Scale of the GZTS) reported higher levels of problems or complaints on nearly all sections, primarily in respiratory, musculo-skeletal, digestive system and fatique. These results indicate that age, unlike neuroticism, does not produce a generalized increase in body concern; instead specific age-related symptoms show increases. Factor analysis of the sections showed two factors: one highly related to age, the other to Neuroticism. No general physical health status factor emerged. These results confirm earlier findings that Neuroticism is an important contributor to self-perceived health, independent of age, and should be controlled in studies which use self-report measures of health status.

G. Results of the first study showed that individual differences in personality were related to latencies in mental performance tasks. Quicker responses were found for more anxious and more open subjects although these results did not hold for all tasks. In the study on visual memory, maintenance of memory performance was predicted by General Activity, Restraint, and Masculinity. Confirmatory results from other studies would encourage the development of intervention strategies capitalizing on these dispositional characteristics.

Significance tc Biomedical Research and the Programs of the Institute:

Psychological stress has long been considered a possible contributor to a number of illnesses; and several interventions have been designed to reduce stress and thus promote health. However, many questions remain about the proposed link. These include the identification of specific illnesses which are stress-related; the length of time between stress and subsequent illness; and the nature of the mechanisms by which psychological states influence physical health. In the past, research on these questions has been hampered by the use of unreliable measures and retrospective designs. The program of research of the Section on Stress and Coping can make substantial contributions to this area by its use of prospective designs and well-validated measures on a large population with documented medical histories. Research on personality may contribute to an understanding of the mechanisms linking stress and illness; at a minimum, it is essential to an understanding of the measurement of stress and illness. Research on stability and change in personality, on the developmental antecedents of Openness to Experience, and on the determinants of well-being and successful aging contribute directly to the mission of this institute.

Proposed Course of the Project

A. New data will be collected to learn if the relations between personality and subjective well-being can be generalized to other samples and measures. The influence of potentially stressful events will be assessed by comparing the well-being levels of groups who have experienced different events. The presence of adaptation level effects will be assessed by comparing the perceived stressfulness of daily events in different age, Socio-economic status, and life events groups. A number of additional measures, including a Coping Style Questionnaire; scales from the Jackson Personality Inventory; the Reigel & Reigel Rigidity Scales; the Crowne-Marlowe Social Desirability Scale; the Hanes General Well-being; an Empathy scale; the Defense Mechanism Inventory; and a biographical history questionnaire will be added to the test battery. In addition, measures of life events and well-being will be re-administered.

B. Data collection will be completed during this year and data analyzed. If predictive relations are found between stress, personality, and psoriasis, the study can be replicated on a larger sample, and possible interventions to modify stressfulness of events will be considered.

C. Data collection will continue with men and begin with women. Protocols will be scored and coding systems will be developed for quantifying information obtained in interviews. Psychiatric interviews to be conducted by a trained psychiatrist will be introduced.

D. Additional evidence on the developmental antecedents of Openness to Experience will be sought by examining data obtained in a biographical questionnaire to determine whether childhood environments or later life events (e.g., "off-time" transitions, occupational shifts, military experience) influence present levels of Openness.

E. The longitudinal analysis of change or stability requires the collection of data over a period of many years. A battery of tests has been selected which will be administered by mail to BLSA participants and their spouses on a cyclical basis. Most tests will be re-administered after six years, at which time longitudinal analyses will be performed. The use of a 10% holdout sample, who will receive these tests for the first time in six years, makes possible the use of cross-sequential and time-sequential designs to distinguish generational and time-of-measurement effects from true maturational changes. In the intervening six years, it will be possible to analyze data from new measures in conjunction with existing data from the GZTS, Activities and Attitudes Questionnaire, and other instruments. While true longitudinal designs cannot be used with different instruments, long-term predictive relations can be sought, and certain kinds of quasi-experimental designs can be used. Specific plans include an analysis of the effects of retirement and widowhood on subjective well-being.

F. Future analyses of the CMI will involve attempts to trace the influence of biomedical variables, such as blood pressure and cardiovascular disease, on self-reported health. These projects will involve collaboration with medical investigators at GRC who have collected other data on BLSA participants. Since self-perceived health is likely to influence the health behaviors of individuals, future studies will also investigate the influence of self-perceived health on perceived vulnerability and such behaviors as self-medications, health habits, and compliance with medical regimens.

G. Over the past years, a variety of cognitive measures have been collected on participants at the Baltimore Longitudinal Study of Aging. Some data on personality has been collected, specifically the GZTS, and much additional data will be gathered by the mailing project of the section. Joint analyses of these data in collaboration with other investigators will allow exploration of many important cognitive functions in relation to personality. In particular, plans are being made to examine the Army Alpha Test and other measures of cognitive functioning.

Publications:

Costa, P. T., Jr., and McCrae, R. R.: Still stable after all these years: Personality as a key to some issues in aging. Life-Span development and behavior, Vol. III. New York, Academic Press, in press.

Costa, P. T., Jr., and McCrae, R. R.: The influence of extraversion and neuroticism on subjective well-being: Happy and unhappy people. Journal of Personality and Social Psychology, in press.

Costa, P. T., Jr., McCrae, R. R., and Arenberg, D.: Enduring dispositions in adult males. Journal of Personality and Social Psychology, in press.

Laboratory of Cellular and Molecular Biology

The Laboratory of Cellular and Molecular Biology is a recently created laboratory, which brings together various research projects that are related through a common interest in the genetic basis for the biological aging process. The Section on Inorganic Biochemistry is concerned with fundamental studies at the molecular level of biological macromolecules involved in genetic information transfer. Age changes are investigated in systems involving these molecules, and particular emphasis is placed on the beneficial, as well as harmful, effects of metal ions that interact with these molecules. The Section on Macromolecules is concerned with the interaction of cells with synthetic macromolecules. Age changes on the cell surface are probed with specially designed macromolecules, and potential polymeric drugs are synthesized. Physico-chemical alterations of chromatin and its transcription are monitored, and age changes in gene expression and in abnormal protein degradation are studied. The Section on Cellular Aging and Genetics is concerned with cell replication and repair of DNA damage as a function of aging. It also deals with the mechanism of parental age effects.

The structure of the nuclear chromatin that is responsible for regulation of genetic activity depends in part on the interactions of the nucleic acids and protein (histone) components. The interactions are characterized by highly intense circular dichroism (CD) spectra. Histones induce positive or negative CD spectra by reaction with DNA; these we call $\psi(+)$ and $\psi(-)$ spectra. Lysine-containing polypeptides produce similar spectra with DNA and other polynucleotides. We have elucidated the forces leading to the $\psi(+)$ and $\psi(-)$ effects by studying the interaction of the synthetic polynucleotides poly d(G-C) and poly d(A-T) with lysine-containing polypeptides. There are actually two types of positive CD effects which we designate $\psi(+)$ and $\psi^*(+)$. Both poly d(G-C) and poly d(A-T) produce a $\psi(-)$ effect when reacted with polylysine in high salt and a $\psi^*(+)$ effect in low salt. Since the secondary structure of the polynucleotides varies with salt concentration, it appears that the different secondary structures lead to different ways of packing the polynucleotides, and it is these packing forms that are responsible for the ψ spectra.

The $\psi(+)$ spectra differ from $\psi^*(+)$ in the position of the CD maximum. The former are induced in polynucleotides by poly(lysine, alanine) copolypeptides: the higher the Ala/Lys ratio, the greater the tendency for $\psi(+)$ formation. However, the amount of alanine required to produce $\psi(+)$ with poly d(G-C) is much less than the amount required with poly d(A-T). This difference between the copolymers can be explained by the greater ease with which the "A" secondary structure can be induced in poly d(G-C), and the "A" structure leads to $\psi(+)$ anisotropy. Thus the formation of $\psi(+)$, $\psi^*(+)$ and $\psi(-)$ forms of aggregated polynucleotides seems to be related to their ability to form polymorphic secondary structures.

Metal ions are important in the packing of chromatin, and they readily alter the ψ characteristics of polynucleotide-polypeptide complexes. The relative ability of various metals to alter the ψ structure can be measured by the alanine content required in a (Lys,Ala) copolypeptide for the metal to induce ψ(+) in DNA. Thus Cu(II) can produce ψ(+) in DNA·polylysine; zinc cannot, but will produce ψ(+) in DNA·poly($Lys_{70}Ala_{30}$). Thus Cu(II) is more effective than zinc in altering the anisotropic packing of a nucleic acid.

Previously described techniques for studying age changes in the accessibility of DNA in rat liver chromatin, using nuclease degradation, have been considerably improved. The result that DNA from old chromatin is more accessible than that from young is corroborated; mean reaction rates for old and young chromatin were 5.05, and 3.69, x 10^{-6} m.eq./ min., respectively, with $p < 0.004$. These results were obtained with relatively "insoluble" chromatin obtained by mild treatment of nuclei. First results with the better characterized "solubilized" chromatin obtained by a method modified from well-known procedures also led to similar results.

Crapper and his associates have obtained evidence that Al accumulates in localized areas of the brain of patients with Alzheimer's disease. The Al is localized in chromatin. Therefore, we have been collaborating with Crapper and Karlik in studies to determine the nature of the interactions of Al with DNA.

The reaction of Al with DNA is different from the reaction of any other previously studied metal ion with DNA in that equilibria exist between several Al-DNA complexes under most conditions. At low pH Al exists mainly in the form of $[Al(H_2O)_6]^{3+}$, and this species crosslinks DNA strands, presumably while losing two molecules of water. This type of Al binding destabilizes the double helix. At high pH the species $[Al(H_2O)_5(OH)]^{2+}$ predominates and stabilizes the double helix by binding to the phosphate groups. At all pH's some DNA molecules bind to both Al species, so that stabilizing and destabilizing tendencies are present simultaneously.

DNA helicity is readily measured with ethidium bromide, which intercalates between the bases of a helix but not those of random-coil strands of DNA. DNA that is unwound by heating at low pH with Al loses the ability to react with ethidium bromide. When the Al is removed by chelation to EDTA, the ethidium bromide again intercalates, thus proving the regeneration of double helix. The fact that the Al crosslinks the DNA strands is also confirmed in this way. Evidently the low pH Al-DNA complex may be expected to have the most dramatic effect on DNA molecules.

Our studies on age changes in ribosome structure and protein synthesis are based on the concept that age changes can be more readily detected when the protein synthesis machinery is under stress. This stress has been conferred in one of two ways: (1) Metal ions directly cause mispairing of bases in the codon-anticodon interaction between messenger RNA and transfer RNA. (2) Antibiotics change ribosomal conformation and in this way indirectly affect the codon-anticodon interaction that is the basis for the correct incorporation of amino acids into protein. Using poly(U) as the message, "correct" and "incorrect" incorporation are phenylalanine and leucine respectively. Ribosomes were prepared from livers of 6-7 month and 23-24 month male rats from the GRC colony; soluble enzymes required in protein synthesis were from 9 month old rat livers.

We found that low Mg^{2+} concentration did favor only "correct" phenylalanine incorporation and higher Mg^{2+} concentration allowed the misincorporation of leucine. The phe optimum occurs at 8 mM Mg^{2+}, and the leu optimum at 11 mM Mg^{2+}. Early results based on 4 young and 6 old samples appeared to show a significant increase in the leu/phe ratio of the old rats ($p < 0.05$) at 11 mM Mg^{2+}. The significance of this increase seems to diminish with a greater number of samples (10-11 for each age group). We now believe that we have no evidence for any age change in fidelity of protein synthesis with ribosomes challenged with Mg^{2+}.

We also stressed ribosomes with the antibiotic, paromomycin, which is one of a class of aminoglycosides found to be effective in eukaryotic systems; paromomycin causes mistranslation both *in vitro* and *in vivo*. We got much higher levels of misincorporation of leucine with phenylalanine by introducing paromomycin than by increasing Mg, up to 0.2 compared to 0.04 with Mg. With paromomycin there appears to be an increase in misincorporation by old ribosomes over the young, and it appears to be statistically reliable. The largest age difference, in leu:phe ratio, occurs in the range of the rather broad leu maximum, 30-60 μM paromomycin; here the leu:phe ratio is about 0.12, and the increase from young to old is about 0.01 (8%), significant at $p < 0.05$.

RNA synthesis mediated by RNA polymerase requires activation by metal ions. The nature of the metal ion determines whether the enzyme (from *E. coli*) permits incorporation of only the correct ribonucleotides or also the incorrect deoxynucleotides. We have therefore studied the effect of Mg^{2+}, Mn^{2+} and Co^{2+}, the three activating metal ions, on the conformation of the enzyme in the presence of DNA template and substrate. Conformational differences are determined from "melting" curves of the enzyme, from which the conformational stability of the enzyme can be ascertained. Surprisingly, it was discovered that the conformational stability is very sensitive to the difference between rATP and dATP in the presence of Mn^{2+} and DNA. There is no such sensitivity with Mg^{2+} or Co^{2+}, or with any of the metal ions in the absence of DNA template. It thus appears that only Mn^{2+} is capable of inducing a conformational change in the enzyme that depends on the presence or absence of a 2'hydroxyl group in the ribose of the nucleoside triphosphate substrate. Mn^{2+}, on the other hand, is the only metal that cannot make this distinction in the incorporation of the substrate into RNA. At present we are puzzled by this anomaly, and we are attempting to resolve it. We hope that NMR studies will shed further light on the problem.

A decline in the proliferative capacities of certain cell populations is an important feature of human aging. Because of the practical and ethical limitations of *in vivo* human experimentation, considerable effort has been expended to create appropriate *in vitro* tissue culture systems as well as *in vivo* animal models for examining age-related alterations in cell replication.

For measurment of cell replication in vivo and in vitro young and old cell populations were incubated with BrdU. At increasing time intervals, animals were sacrificed or cell cultures harvested and chromosomal preparations were made. Cells that had undergone 1, 2 and 3 cell cycles in the presence of this drug could be clearly identified by characteristic banding patterns. By utilizing the BrdU-differential staining technique both in vitro and in vivo, one can measure the frequency of sister chromatid exchanges (SCE) in second replication cycle cells.

Examination of phytohemagglutinin (PHA) stimulated human peripheral lymphocytes indicated that with aging there is a decline in the number of cells responding to PHA as well as a reduction in the replication rate of the PHA responsive cell populations. Investigation of mutagen induction of SCE in skin fibroblast cultures derived from young and old human volunteers demonstrated a decrease in SCE induction at high mutagen levels in old cell populations. Analysis of mutagen induction of SCE in immature and mature mice and rats revealed decreased SCE formation at high mutagen concentrations in immature animals. Thus mutagen-induced SCE levels increase with post-embryonic development, plateau during maturity and then decline with aging. The frequency of baseline or spontaneous SCE was measured as a function of in vitro serial passage in IMR-90 fetal lung fibroblasts. No significant alteration in spontaneous SCE levels was observed.

The in vivo analysis of SCE has been extended to examine the induction of SCE in utero in mice. By infusing BrdU and the mutagens to be tested, SCE induction can be examined concurrently in both fetal and maternal tissues permitting analysis of transplacental transport of mutagens. Our results indicate that exposure to teratogens which act through DNA damage can be detected at drug concentrations well below that which are teratogenic.

In vivo BrdU-differential chromatid staining techniques were also applied to examine the effect of different cancer chemotherapeutic agents. Mice with transplanted Ehrlich Ascites tumors were infused with BrdU and the agent to be tested. Both normal bone marrow and Erhlich Ascites tumor cell populations were then analyzed for SCE induction and for inhibition of cellular replication kinetics. This permits the examination of differential toxicity of these compounds on normal and tumor cell populations.

Recent evidence indicates that advanced paternal age may contribute to the increased frequency of chromosomally abnormal offspring with advanced parental age. This paternal age component can be directly evaluated by examining the chromosomal complement of human sperm samples derived from volunteer members of the Baltimore Longitudinal Study.

There appears to be a significant increase in the frequency of double Y bodies as a function of paternal age. Flow microfluorometric analyses indicate that this technique can be applied to examining chromosomal aneuploidy in human sperm samples. Results obtained by flow microfluorometry appear to correlate well with staining for Y bodies.

The cell surface is constantly damaged by oxygen and this damage must be continuously repaired. In order to establish whether there is an age decline in this repair the corresponding analytical method was worked out and applied to postmitotic cells of different *in vivo* age and to non-aging transformed cells. An impermeant reagent, a dextran of high molecular weight with covalently attached mercury atoms, was used for this purpose. Mercury-dextran was used in the form of a cysteine complex; it binds to multiple sulfhydryl groups by a reaction which releases cysteine. The reaction between cell surface sulfhydryl groups and mercury-dextran is selective since (a) the binding of the reagent to cells is cancelled when mercury is eliminated from mercury-dextran by reducing it with sodium borohydride, (b) the binding of mercury-dextran to cells is considerably decreased by the irreversible blocking of cellular sulfhydryl groups with N-ethylmaleimide and (c) the mild reduction of viable cells, which converts surface disulfide groups into sulfhydryl ones increases considerably the binding of the reagent.

Adipocytes isolated from Wistar and Sprague-Dawley strains of rats of different ages were used as postmitotic cells. The binding of mercury-[^{3}H] dextran to native or pre-reduced cells was measured. Aging was found to significantly decrease the ratio of the number of exofacial sulfhydryl groups to the total number of exofacial sulfhydryl and disulfide groups.

Mouse erythroleukemic cells were used as non-aging cells. Accessible sulfhydryl groups on the surface bind 9 amoles of organic mercury per cell and during the 18-month subcultivation of cells this number did not change significantly. This number is increased up to 38 after reduction of cell surface disulfides and did not change with subcultivation of cells. The reduction was achieved without any significant loss of cellular protein and without any increase in cell permeability to a small molecular weight compound. Treatment of cells with a macromolecular reducing agent, dextran substituted with sulfhydryl groups, did not lead to any increase in the number of surface sulfhydryl groups. Thus there are only a few reducible disulfide groups located in unexposed positions in proteins present on the cell surface and can be reduced with an accompanying unfolding of the protein structure but without release of these proteins from the cells.

The aging of fibroblasts *in vitro* leads to a significant proportion of cells with an apparently large surface area which grow only to relatively low densities. On the other hand, after viral transformation the fibroblasts grow to very high densities and the cells have an apparently low surface area. A macromolecular probe was used to compare clustered anionic sites, which form an important part of the surface in normal, senescent and transformed cells. The binding of polyuridylate in the presence of proflavine was used for that purpose. It was established that this probe binds only to the external surface of cells to clustered carboxylate groups and O- or N-hemisulfate groups there. Virus transformed human fibroblasts bound ten times less of the probe than non-transformed cells when confluent monolayers were compared. However, the amount of probe bound per cell decreased strikingly as the cell density was increased both for transformed and normal cells. At the same cell density human fibroblasts (a) derived from normal donors of different ages, (b) from donors with different metabolic disorders and (c) from cells transformed by simian virus 40, all bind about the same amount of probe. Thus, cells which have distinct morphological and biochemical differences, when compared at the same cell density, have the same amount of the surface binding sites for this probe. However, populations of human fibroblasts aged in vitro and containing high proportions of large cells bound disproportionately large amounts of the probe.

Daunorubicin, a chemotherapeutic agent currently used in the treatment of leukemia, was bound by a hydrolyzable bond, via its keto group, to macromolecular hydrazides. These compounds were somewhat less cytotoxic to mouse lymphoma cells (Yac) in vitro but were equally or more effective against the Yac lymphoma in mice. The macromolar part of the hydrazides were derived from carboxymethyldextran, polyglutamate, alginic acid and hydroxymethylcellulose. Daunorubicin was also attached to a carrier by a non-hydrolyzable sulfide bond, by condensation of 14-bromodaunorubicin with 3-mercapto-2-hydroxypropyldextran. This compound did not have either cytotoxic effects in vitro or antitumor effects in vivo. Macromolecules containing daunorubicin bound by hydrazone linkage were attached to goat anti-Yac IgG; these conjugates were found to gradually lose their solubility.

Studies of the possible age-dependent relaxation of specific gene expression in tissues where the presence of these genes is not expected have continued. These studies have now included the structural genes: α and β globin, casein and α-fetoprotein, and the endogenous C-type viruses: mouse leukemia virus (MuLV) and mouse mammary tumor virus (MMTV). Both globin and α-fetoprotein RNA appear to increase in nuclei and cytoplasm of the liver for the long-lived C57BL/6J mouse strain and globin RNA in the nuclei and cytoplasm of the brain for this strain. Of particular interest is the high fraction of globin RNA found only in the liver nuclei of the old AKR mouse strain. This mouse strain has a high incidence of leukemia. A high age-dependent increase in MuLV-RNA in thymus but not in liver and brain was also found for the AKR strain. Although MuLV-RNA was found to increase with age in the nuclei and cytoplasm of liver and brain in the long-lived C57BL/6J mouse strain, MMTV-RNA was found to increase only in liver nuclei. Globin RNA also appears to increase with age in human brain, as was previously found to occur in brain tissue of the mouse.

An age-dependent increase in improper gene expression could result from epigenetic and/or mutational-like changes in the genetic apparatus of cells. We have continued to investigate the T-3 hormone binding capacity and affinity to chromatin and whole nuclei in rat liver as a function of age. There is still an indication that an age-dependent decrease in binding capacity occurs, although considerable fluctuation is found. Little change with age in the binding constants were apparent. We have also investigated the T-3 hormone binding capacity in old hypophysectomized rats. These animals have undergone an apparent reversal in a number of immunological parameters and kidney function, approaching that of a young animal. However, no change was found in the low T-3 binding capacity typical for the old non-hypophysectomized animals. However these old hypophysectomized rats were found to undergo a remarkable reversal in a number of morphological parameters of the kidney, as determined by electron microscopy.

Correlations are sought between levels of potential genetic repair and protective processes and the aging rate of the mammalian species.

Work continued on the superoxide dismutase (SOD) and guanylate cyclase specific activity studies using brain, liver and heart tissues of two rodent and twelve primate species. These species covered a maximum lifespan potential (MLP) range of 3.5 to 95 years. No correlation was found between the level of SOD specific activity and MLP. However, an excellent correlation was found between the ratio of SOD and specific metabolic rate. Preliminary data with catalase show similar results. These results suggest that longer lived species have superior protection against oxygen toxicity. Measurement of guanylate cyclase (GC) specific activity for the same species indicates an inverse correlation with SOD specific activity. In view of the activation of GC by hydroxyl radicals, these results suggest that GC specific activity may be involved in governing the level of SOD specific activity and perhaps other intracellular free radical protective processes.

We have previously found that zinc binds to hemoglobin producing a three-four fold increase in oxygen affinity. In order to relate this effect to a possible in vivo effect of zinc we are investigating the effect of zinc on intact erythrocytes. The addition of $ZnCl_2$ to erythrocytes results in agglutination of the cells and 0.15-0.17 moles of zinc per heme associated with the erythrocyte. The location of this zinc predominantly on the outside of the erythrocyte is indicated by the ability to remove the bulk of this zinc with EDTA and the decrease in the amount bound after pronase treatment. Zinc can be transported through the membrane to the inside of the cell when ionophore A-23187 is incorporated into the membrane. This ionophore binds divalent metal ions producing a channel in the membrane through which these divalent ions can pass. When this is done, the cellular concentrations of zinc can be even higher than that of the heme. Most of this zinc cannot be removed by complexing agents; therefore it is probably inside the cell. Since the concentration of zinc incorporated in the cell is higher than the one zinc for every two hemes found to bind to purified hemoglobin solutions, there must be additional binding sites on the inner surface of the membrane and/or to other molecules in the cytoplasm.

Interactions of Ca^{2+} and Pb^{2+} with the erythrocyte membrane have previously been indicated. Intracellular Ca^{2+}, which is generally kept in the μM range by an ATPase which pumps the Ca^{2+} out, has been shown to alter the deformability of the erythrocyte and produce a number of other deleterious effects, when the intracellular Ca^{2+} level increases. We have compared the binding of Ca^{2+}, Pb^{2+}, and Zn^{2+} to erythrocyte ghosts as well as to the proteins removed from the membrane at low ionic strength. All three metal ions bind to the open ghosts with Ca^{2+} having a lower affinity than the other two metal ions. On the other hand, Pb^{2+}, but not Zn^{2+}, seems to produce many of the same membrane effects as Ca^{2+}. In fact, Zn^{2+} seems to reverse some of the deleterious effects of Ca^{2+} and Pb^{2+}.

We have previously found that the rate of hemolysis decreases with increasing age, and that this decrease in rate is associated with an increase in the membrane cholesterol level. Since cholesterol is known to decrease mobility of the lipid matrix, these results suggested a possible relationship between the mobility of membrane components and the rate of hemolysis. In order to test this hypothesis we have looked at the effect on the rates of hemolysis of a number of different membrane modifications which alter the mobility of different components of the membrane and, at the same time, we have used spin labels in order to look directly at the effects of these modifications on the mobility of the membrane components. As a result of these studies, we find that changes in the mobility of proteins which frequently go together with a decrease in deformability do not correlate with the rate of hemolysis. However, changes in lipid fluidity do correlate with the rate of hemolysis. The decrease in rates of hemolysis with increasing age, therefore, suggests a change in erythrocyte membrane fluidity for older individuals and the possibility of important alterations in fluidity of the membrane with age.

We have previously found that horse hemoglobin is much more readily oxidized than human hemoglobin by low Cu(II) concentrations. This difference was shown to be associated with an additional high affinity site on human hemoglobin from which Cu(II) cannot oxidize the iron of hemoglobin. Hemoglobin A_{1_c} is the result of the post translational reaction of glucose with hemoglobin and has a glucose molecule attached to the terminal amino groups of the β-chains of human hemoglobin A_o. Interestingly, this single modification is found to alter the interaction of hemoglobin with Cu(II) so that hemoglobin A_1 is more similar to horse hemoglobin than human hemoglobin with respect to its reactions with copper.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00044-06 LCMB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Metals and Proteins on Nucleic Acids, Information Transfer, and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Section on Inorganic Biochemistry

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.4	4.6	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project focuses on the interaction of molecules concerned with genetic information transfer. A primary objective is to determine under what conditions metal ions are essential for information transfer, and under what conditions they produce errors in the information and may thus contribute to biological aging. Topics of interest are: (1) the effects of metal ions on the structure of nucleic acids, nucleoproteins and chromatin; (2) age-related changes in chromatin structure; (3) aging of ribosomes; (4) the mechanism of involvement of aluminum in Alzheimer's disease; (5) crosslinking of nucleic acid strands by metal ions; (6) the effects of metal ions on RNA polymerase; (7) metal ions and cellular aging.

Project Description:

Objectives: (1) To study effects of metal ions on the structures involved in the replication, transcription, and translation of genetic information transfer, (2) to determine how errors can be introduced into genetic information transfer by metal ions, and how these errors may affect aging, (3) to understand the interactions of proteins and nucleic acids and their mediation by metal ions, (4) to understand the role of metal ions in toxicity and aging, (5) to understand the participation of metal ions in the biological activities of nucleic acids and nucleoproteins, (6) to elucidate structural and functional changes in genetic information transfer that accompany aging.

Methods Employed: (1) The interaction of metal ions, proteins, and nucleic acids are studied by optical rotatory dispersion, circular dichroism, and uv spectrophotometry to determine conformational changes, and by infrared, nuclear magnetic resonance, and electron spin resonance techniques to determine interaction sites. (2) Chromatin structure is analyzed by means of model systems to determine which structural features are responsible for its function. (3) Physical chemical techniques are employed to study age changes in chromatin. (4) Effects of metal ions on nucleic acids and nucleoprotein are studied to determine under what conditions they serve an essential function in information transfer, and under what conditions they induce errors in information content. (5) The effect of metal ions on the enzymes responsible for genetic information transfer are studied. (6) The mechanisms by which enzymes and metal ions synthesize and degrade nucleic acids are elucidated. (7) Age changes in ribosomal fidelity are investigated by stressing the ribosomes with high concentrations of metal ions and with antibiotics.

Major Findings:

A. The reaction between polynucleotides and polypeptides. The structure of the nuclear chromatin that is responsible for regulation of genetic activity depends in part on the interactions of the nucleic acids and protein (histone) components. The interactions are characterized by highly intense circular dichroism (CD) spectra. Histones induce positive or negative CD spectra by reaction with DNA; these we call $\psi(+)$ and $\psi(-)$ spectra. Lysine-containing polypeptides produce similar spectra with DNA and other polynucleotides. We have elucidated the forces leading to the $\psi(+)$ and $\psi(-)$ effects by studying the interaction of the synthetic polynucleotides poly d(G-C) and poly d(A-T) with lysine-containing polypeptides. There are actually two types of positive CD effects which we designate $\psi(+)$ and $\psi^*(+)$.

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The $\psi(+)$ spectra differ from $\psi^{*}(+)$ in the position of the CD maximum. The former are induced in polynucleotides by poly(lysine, alanine) copolypeptides: the higher the Ala/Lys ratio, the greater the tendency for $\psi(+)$ formation. However, the amount of alanine required to produce $\psi(+)$ with poly d(G-C) is much less than the amount required with poly d(A-T). Poly d(G-C) forms $\psi(+)$ complexes with poly($Lys_{70}Ala_{30}$), poly($Lys_{50}Ala_{50}$) and poly($Lys_{30}Ala_{70}$), poly d(A-T) forms $\psi(+)$ with poly($Lys_{30}Ala_{70}$) only. This difference between the copolymers can be explained by the greater ease with which the "A" secondary structure can be induced in poly d(G-C), and the "A" structure leads to $\psi(+)$ anisotropy. Thus the formation of $\psi(+)$, $\psi^{*}(+)$ and $\psi(-)$ forms of aggregated polynucleotides seems to be related to their ability to form polymorphic secondary structures.

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"Melting" curves of "insoluble" chromatin showed cooperative melting, but age changes in the melting temperature and the hyperchromicity were not statistically significant. CD age changes in the chromatin were also not significant. So far only the accessibility studies have led to detectable age changes.

We are studying methods of stressing the rat liver chromatin with metal ions, not only to determine the effects of these ions on chromatin, but in the hope that age changes in the chromatin can be more readily detected under stress. Metal ions generally bring about the aggregation of chromatin.

C. Reaction of aluminum with DNA. Crapper and his associates have obtained evidence that Al accumulates in localized areas of the brain of patients with Alzheimer's disease. The Al is localized in chromatin. Therefore, we have been collaborating with Crapper and Karlik in studies to determine the nature of the interactions of Al with DNA.

The reaction of Al with DNA is different from the reaction of any other previously studied metal ion with DNA in that equilibria exist between several Al-DNA complexes under most conditions. At low pH Al exists mainly in the form of $[Al(H_2O)_6]^{3+}$, and this species crosslinks DNA strands, presumably while losing two molecules of water. This type of Al binding destabilizes the double helix. At high pH the species $[Al(H_2O)_5(OH)]^{2+}$ predominates, and stabilizes the double helix by binding to the phosphate groups. At all pH's some DNA molecules bind to both Al species, so that stabilizing and destabilizing tendencies are present simultaneously.

DNA helicity is readily measured with ethidium bromide, which intercalates between the bases of a helix but not those of randon-coil strands of DNA. DNA that is unwound by heating at low pH with Al loses the ability to react with ethidium bromide. When the Al is removed by chelation to EDTA, the ethidium bromide again intercalates, thus proving the regeneration of double helix. The fact that the Al crosslinks the DNA strands is also confirmed in this way. Evidently the low pH Al-DNA complex may be expected to have the most dramatic effect on DNA molecules.

D. Age changes in fidelity of protein synthesis. Expression of the genetic information encoded by nucleic acids into proteins is basically determined by the pairing of messenger RNA (codon) and amino acid transfer RNA (anticodon) bases. But the protein synthetic system, the focus of which is the ribosome, modulates the specificity of the codon-anticodon interaction with the result that the frequency of incorrect amino acid insertion is not determined by codon-anticodon base pairing alone. The ribosome, comprising a complex of several RNA chains and numerous protein units, provides an assemblage of sites for binding and manipulating the mRNA, tRNA and the nascent protein chain, for various enzyme reactions, and also for interchange of several protein units which facilitate or control transfer of reagents and products or otherwise function as effectors.

The fidelity of protein synthesis, controlled as it is by the ribosome, can be altered by changes in the ribosome. Also, fidelity can deteriorate under various kinds of stress involving the ribosome: by increased magnesium concentration decreasing the inherent specificity of codon-anticodon base pairing, by introduction of an antibiotic which directly affects the ribosome by binding to a site, or even by a deficiency in the aminoacyl form of the tRNA ordinarily specified by a particular codon. Because ribosomes of various species show differing ability to maintain fidelity in the face of the stress of high Mg or introduction of antibiotics, we have undertaken to use these stresses to detect possible age changes in the capacity of the ribosome to maintain fidelity.

Ribosomes are prepared from livers of 6-7 month and 23-24 month virgin male rats from the GRC colony; soluble enzymes are prepared from livers of 9 month rats. The preparative methods are designed to produce monoribosome samples of overall high yield, representing both membrane-bound and nonmembrane-bound polysomes, from which very little of the constitutive protein components have been lost; and a mixture of soluble proteins as a source of elongation factors which may be required. At present we use the simple model of coincorporation of leucine (codons: CUA/G/UC, UUA/G) with phenylalanine (codons: UUU/C) on ribosomes programmed with poly(U) as message; misreading of one base out of three permits the incorporation of leu in place of phe. Incorporation of both amino acids is measured simultaneously by a double label method. In order to avoid effects of depletion of phe-acylated $tRNA^{phe}$ and leu-acylated $tRNA^{leu}$, we introduce known amounts of the acylated tRNA species and allow their regeneration. To assure that incorporation of phe and leu is not being altered by changing conditions, we measure rates of incorporation and restrict measurement to the first linear phase of the reaction.

We found that low Mg^{2+} concentration did favor only "correct" phenylalanine incorporation and higher Mg^{2+} concentration allowed the misincorporation of leucine. The phe optimum occurs at 8 mM Mg^{2+}, and the leu optimum at 11 mM Mg^{2+}. Early results based on 4 young and 6 old samples appeared to show a significant increase in the leu/phe ratio of the old rats ($p < 0.05$) at 11 mM Mg^{2+}. The significance of this increase seems to diminish with a greater number of samples (10-11 for each age group). We now believe that we have no evidence for any age change in fidelity of protein synthesis with ribosomes challenged with Mg^{2+}.

We also stressed ribosomes with the antibiotic, paromomycin, which is one of a class of aminoglycosides found to be effective in eukaryotic systems; paromomycin causes mistranslation both _in vitro_ and _in vivo_. We got much higher levels of misincorporation of leucine with phenylalanine by introducing paromomycin than by increasing Mg, up to 0.2 compared to 0.04 with Mg. With paromomycin there appears to be an increase in misincorporation by old ribosomes over the young, and it appears to be statistically reliable. However, at this stage in our investigations we cannot discern age changes in the phe or leu responses by themselves, due to experimental scatter; the scatter is effectively cancelled in the leu:phe ratio, and much more noticeably so than in the case of the Mg experiments. The largest age difference, in leu:phe ratio, occurs in the range of the rather broad leu maximum, 30-60 μM paromomycin; here the leu:phe ratio is about 0.12, and the increase from young to old is about 0.01 (8%), significant at $p < 0.05$.

E. _The effect of metal ions on RNA polymerase_. RNA synthesis mediated by RNA polymerase requires activation by metal ions. The nature of the metal ion determines whether the enzyme (from _E. coli_) permits incorporation of only the correct ribonucleotides or also the incorrect deoxynucleotides. We have therefore studied the effect of Mg^{2+}, Mn^{2+} and Co^{2+}, the three activating metal ions, on the conformation of the enzyme in the presence of DNA template and substrate. Conformational differences are determined from "melting" curves of the enzyme, from which the conformational stability of the enzyme can be ascertained. Surprisingly, it was discovered that the conformational stability is very sensitive to the difference between rATP and dATP in the presence of Mn^{2+} and DNA. There is no such sensitivity with Mg^{2+} or Co^{2+}, or with any of the metal ions in the absence of DNA template. It thus appears that only Mn^{2+} is capable of inducing a conformational change in the enzyme that depends on the presence or absence of a 2'hydroxyl group in the ribose of the nucleoside triphosphate substrate. Mn^{2+}, on the other hand, is the only metal that cannot make this distinction in the incorporation of the substrate into RNA.

At present we are puzzled by this anomaly, and we are attempting to resolve it. We hope that NMR studies will shed further light on the problem.

Significance to Biomedical Research and to the Program of the Institute: The studies on the age changes in chromatin are of obvious relevance. The participation of metal ions in every aspect of genetic information transfer and the deleterious effects on this transfer caused by undesired metal ions or essential metal ions in undesired concentrations make the study of metal ion interactions with nucleic acids of major importance. The possible relationship between aluminum accumulation and Alzheimer's disease and the discovery that the aluminum is bound to chromatin have emphasized the importance of studies on metal interaction with nucleic acids and chromatin. An understanding of the structure and function of chromatin (and therefore protein - DNA interaction), ribosomes, the nucleic acid polymerases, etc. is essential to an understanding of cellular aging. We are particularly interested in studies that show how information transfer can go wrong. Metal ions are presumably not responsible for the primary events that cause aging but we believe that they may be important factors in determining individual and geographic differences in the aging process.

Proposed Course: We hope to better understand the structure of the Al-DNA complex from NMR studies, which we hope will clarify the exact binding modes between Al and DNA.

NMR techniques will also be used to determine the nature of the binding between Co^{2+}, RNA polymerase, and ribonucleoside and deoxynucleoside triphosphate substrates, and to compare these results with similar studies with Mg^{2+}. In this way it is hoped to learn why Mn^{2+} causes errors in RNA synthesis and Co^{2+} does not.

Studies on age changes in ribosomes will continue with different protein synthesizing systems, and we shall attempt to analyze the protein products to determine directly the changes brought about by error incorporation.

Age changes in chromatin will be investigated by stressing the chromatin with metal ions. At the same time we shall continue to study the equilibria between nucleic acids, proteins, and metal ions. An understanding of these equilibria is basic to an understanding of the age changes.

We hope to be able to initiate a study on the relationship between the accumulation of Ni^{2+} ions, aging and cataracts. There is some very preliminary evidence that nickel accumulation may be related to cataracts.

Publications:

Srivastava, R.C., Froehlich, J., and Eichhorn, G.L.: The effect of platinum binding on the structure of DNA and its function in RNA synthesis. Biochimie 60: 879-891, 1978.

Eichhorn, G.L., : Aging, genetics and the environment: Potential of errors introduced into genetic information transfer by metal ions. Mech. Ageing Dev. 9: 291-301, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00046-09 LCMB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Polymers as Biological Reagents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	M. Akashi	Visiting Fellow	LCMB NIA (DOD 4/23/79)
	S. Zawadzki	Visiting Fellow	LCMB NIA (EOD 4/23/79)
	J. Zjawiony	Visiting Fellow	LCMB NIA (EOD 9/30/78)

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Section on Macromolecules

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 3.8	PROFESSIONAL: 2.8	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this project chemically reactive and water soluble macromolecules have been synthesized and their effects on mammalian cells in culture studied, mainly emphasizing the study of their interaction with the cell membrane. Polysaccharides, dextran, plant gums and modified peptides, which are neither bound nor consumed by cells, were used as carriers for various chemically reactive groups. The resulting macromolecules have been tested in vitro on mouse erythroleukemic cells and on human fibroblasts.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Macromolecules have only limited potential to penetrate into the inside of cells; thus they represent suitable reagents for the cell surfaces. The cellular surface is an ideal location for drug action; if a suitable macromolecular, cell selective drug can be obtained, a pharmacological intervention can be made without saturating the whole cellular space with the penetrating drug. The ultimate intent of the project is to create a rational basis for the design of such drugs. In these early stages the objective of research is to obtain biologically active macromolecules and correlate their activity with their structure.

Methods Employed: The study requires both chemical and biological methods. Methods for attachment of small molecular weight compounds to polysaccharides were investigated and the stability of the attachment measured under physiological conditions. These compounds were then tested on (a) mouse erythroleukemic cells which grow in suspension and thus are easily obtainable in bulk and (b) human fibroblast cells, which grow in monolayers and are thus obtainable only in smaller amounts.

Major Findings: Cell Surface: Sulfhydryl and Disulfide Groups. The cell surface is constantly damaged by oxygen and this damage must be continuously repaired. In order to establish whether there is an age decline in this repair the corresponding analytical method was worked out and applied to post-mitotic cells of different *in vivo* age and to non-aging transformed cells. An impermeant reagent, a dextran of high molecular weight with covalently attached mercury atoms, was used for this purpose. Mercury-dextran was used in the form of a cysteine complex; it binds to multiple sulfhydryl groups by a reaction which releases cysteine. The reaction between cell surface sulfhydryl groups and mercury-dextran is selective since (a) the binding of the reagent to cells is cancelled when mercury is eliminated from mercury-dextran by reducing it with sodium borohydride, (b) the binding of mercury-dextran to cells is considerably decreased by the irreversible blocking of cellular sulfhydryl groups with N-ethylmaleimide and (c) the mild reduction of viable cells which converts surface disulfide groups into sulfhydryl ones increases considerably the binding of the reagent.

Adipocytes isolated from Wistar and Sprague-Dawley strains of rats of different ages were used as post-mitotic cells. The binding of mercury-[^{3}H] dextran to native or pre-reduced cells was measured. Aging was found to significantly decrease the ratio of the number of exofacial sulfhydryl groups to the total number of exofacial sulfhydryl and disulfide groups.

Mouse erythroleukemic cells were used as non-aging cells. Accessible sulfhydryl groups on the surface bind 9 amoles of organic mercury per cell and during the 18-month subcultivation of cells this number did not change significantly. This number is increased up to 38 after reduction of cell surface disulfides and did not change with subcultivation of cells. The reduction was achieved without any significant loss of cellular protein and without any increase in cell permeability to a small molecular weight compound. Treatment of cells with a macromolecular reducing agent, dextran substituted with sulfhydryl groups, did not lead to any increase in the number of surface sulfhydryl groups. Thus there are only a few reducible disulfide groups located in unexposed positions in proteins present on the cell surface and can be reduced with an accompanying unfolding of the protein structure but without release of these proteins from the cells.

Cell Surface: Clustered Anionic Sites. The aging of fibroblasts *in vitro* leads to a significant proportion of cells with an apparently large surface area which grow only to relatively low densities. On the other hand, after viral transformation the fibroblasts grow to very high densities and the cells have an apparently low surface area. A macromolecular probe was used to compare clustered anionic sites, which form an important part of the surface in normal, senescent and transformed cells. The binding of polyuridylate in the presence of proflavine was used for that purpose. It was established that this probe binds only to the external surface of cells to clustered carboxylate groups and O-or N-hemisulfate groups there. Virus transformed human fibroblasts bound ten times less of the probe than non-transformed cells when confluent monolayers were compared. However, the amount of probe bound per cell decreased strikingly as the cell density was increased both for transformed and normal cells. At the same cell density human fibroblasts (a) derived from normal donors of different ages, (b) from donors with different metabolic disorders and (c) from cells transformed by simian virus 40, all bind about the same amount of probe. Thus, cells which have distinct morphological and biochemical differences, when compared at the same cell density, have the same amount of the surface binding sites for this probe. However, populations of human fibroblasts aged *in vitro* and containing high proportions of large cells bound disproportionately large amounts of the probe.

Cytotoxic Macromolecules. Daunorubicin, a chemotherapeutic agent currently used in the treatment of leukemia, was bound by a hydrolyzable bond, via its keto group, to macromolecular hydrazides. These compounds were somewhat less cytotoxic to mouse lymphoma cells (Yac) *in vitro* but were equally or more effective against the Yac lymphoma in mice. The macromolar part of the hydrazides were derived from carboxymethyldextran, polyglutamate, alginic acid and hydroxymethylcellulose. Daunorubicin was also attached to a carrier by a non-hydrolyzable sulfide bond, by condensation of 14-bromodaunorubicin with 3-mercapto-2-hydroxypropyldextran. This compound did not have either cytotoxic effects *in vitro* or antitumor effects *in vivo*. Macromolecules containing daunorubicin bound by hydrazon linkage were attached to goat anti-Yac IgG; these conjugates were found to gradually loose their solubility.

Significance to Biomedical Research and Program of the Institute. Macromolecules differ profoundly from low molecular weight compounds in their biological potentials. The present studies aim to use these differences in the investigation of the cell surface, a cellular component which is supposed to reflect closely the state and age of the cell itself.

Proposed Course of the Project. By directed synthesis and study of the basic biological effects of macromolecules, it is hoped to gain the knowledge necessary for the design of practical, useful compounds; e.g., reagents that react with the cell surface and specific groups on the cell surface, reagents that can detect surface charge and therapeutically useful macromolecules.

Publications:

Berger, N.A., Kociolek, K. and Pitha, J.: Steric factors in lymphocyte stimulation by organomercurials. Biochem. Biophys. Res. Commun. 86: 1234-1240, 1979.

Caron, M.G., Srinivasan, Y., Pitha, J., Kociolek, K. and Lefkowitz, R.J.: Affinity chromatography of the β-adrenergic receptor. J. Biol. Chem. 254: 2923-2927, 1979.

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Noronha-Blob, L. and Pitha, J.: Binding of polynucleotides to fibroblasts. Effects of complex formation with vinyl analogs of nucleic acids. Biochim. Biophys. Acta 519: 285-290, 1978.

Pitha, J., Kociolek, K. and Apffel, C.A.: Opposite effects of dextrans substituted with sulfhydryls or mercury on tumor growth. Cancer Res. 39: 170-173, 1979.

Pitha, J., Kociolek, K. and Caron, M.G.: Detergents linked to polysaccharides: preparation and effects on membranes and cells. Eur. J. Biochem. 94: 11-18, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00047-09 LCMB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Relation of Structure and Function in Hemoglobin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. M. Rifkind	Research Chemist	LCMB NIA
OTHER:	K. Araki	Visiting Fellow	LCMB NIA (DOD 2/10/79)
	J. T. Wang	Research Chemist	LCMB NIA (EOD 5/1/79)

COOPERATING UNITS (if any)
Duke University Marine Laboratory; Johns Hopkins University; University of Minnesota

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Section on Inorganic Biochemistry

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.25	1.75	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the mechanisms involved in regulating the binding of oxygen to hemoglobin and the transport of oxygen to the tissues. The project also focuses on ways in which these functions are impaired and change with age. We have also studied the mechanisms involved in the oxidation of hemoglobin. Oxidation effects oxygen transport because it produces non-functional hemoglobin, which no longer binds oxygen. We have extended these studies to include an investigation of the stability of the erythrocyte as measured by osmotic fragility and rates of hemolysis. Changes in these parameters have been observed as a function of age and studies have been initiated to explain the origin of these age-related changes.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: (1) To study the binding of ligands to hemoglobin, and the role of the protein in controlling this function. (2) To study the mechanisms for maintaining hemoglobin in its functional form. (3) To study the mechanisms involved in regulating the transport of oxygen to the tissues. (4) To elucidate age-related changes in the composition and functional properties of the erythrocyte.

Methods Employed: Various preparative procedures are used to purify hemoglobin and to separate various components of the erythrocyte. Visible, uv and atomic absorption spectroscopy, as well as gel electrophoresis, are used to analyze for various erythrocyte components. The oxygenation and oxidation of hemoglobin solutions and whole erythrocytes are investigated under various conditions with and without the addition of various substances. Binding of metal ions and other small substances are studied by equilibrium dialysis. Electron spin resonance is used to observe no-spin labels and paramagnetic Cu(II). The extent of hemolysis and the rate of hemolysis of various samples of red cells are observed under various conditions with and without the addition of various substances.

Major Findings:

A. The interaction of zinc with intact erythrocyte. We have previously found that zinc binds to hemoglobin producing a three-four fold increase in oxygen affinity. In order to relate this effect to a possible *in vivo* effect of zinc we are investigating the effect of zinc on intact erythrocytes. The addition of $ZnCl_2$ to erythrocytes results in agglutination of the cells and 0.15-0.17 moles of zinc per heme associated with the erythrocyte. The location of this zinc predominantly on the outside of the erythrocyte is indicated by the ability to remove the bulk of this zinc with EDTA and the decrease in the amount bound after pronase treatment. Zinc can be transported through the membrane to the inside of the cell when ionophore A-23187 is incorporated into the membrane. This ionophore binds divalent metal ions producing a channel in the membrane through which these divalent ions can pass. When this is done, the cellular concentrations of zinc can be even higher than that of the heme. Most of this zinc cannot be removed by complexing agents; therefore it is probably inside the cell. Since the concentration of zinc incorporated in the cell is higher than the one zinc for every two hemes found to bind to purified hemoglobin solutions, there must be additional binding sites on the inner surface of the membrane and/or to other molecules in the cytoplasm.

B. The interaction of metal ions with erythrocyte membranes. Our studies with zinc indicate that zinc interacts with the erythrocyte membrane. Interactions of Ca^{2+} and Pb^{2+} with the erythrocyte membrane have previousl been indicated. Intracellular Ca^{2+}, which is generally kept in the μM range by an ATPase which pumps the Ca^{2+} out, has been shown to alter the deformability of the erythrocyte and produce a number of other deleterious effects, when the intracellular Ca^{2+} level increases. We have compared the binding of Ca^{2+}, Pb^{2+}, and Zn^{2+} to erythrocyte ghosts as well as to the proteins removed from the membrane at low ionic strength. All three metal ions bind to the open ghosts with Ca^{2+} having a lower affinity than the other two metal ions. On the other hand, Pb^{2+}, but not Zn^{2+}, seems to produce many of the same membrane effects as Ca^{2+}. In fact, Zn^{2+} seems to reverse some of the deleterious effects of Ca^{2+} and Pb^{2+}.

C. Membrane bilayer fluidity and the rate of hemolysis.We have previously found that the rate of hemolysis decreases with increasing age, and that this decrease in rate is associated with an increase in the membrane cholesterol level. Since cholesterol is known to decrease mobility of the lipid matrix, these results suggested a possible relationship between the mobility of membrane components and the rate of hemolysis. In order to test this hypothesis we have looked at the effect on the rates of hemolysis of a number of different membrane modifications which alter the mobility of different components of the membrane and, at the same time, we have used spin labels in order to look directly at the effects of these modifications on the mobility of the membrane components. As a result of these studies, we find that changes in the mobility of proteins which frequently go together with a decrease in deformability do not correlate with the rate of hemolysis. However, changes in lipid fluidity do correlate with the rate of hemolysis. The decrease in rates of hemolysis with increasing age, therefore, suggests a change in erythrocyte membrane fluidity for older individuals and the possibility of important alterations in fluidity of the membrane with age.

D. A comparison of the interaction of Cu(II) with human hemoglobin A_o and A_{1_c}. We have previously found that horse hemoglobin is much more readily oxidized than human hemoglobin by low Cu(II) concentrations. This difference was shown to be associated with an additional high affinity site on human hemoglobin from which Cu(II) cannot oxidize the iron of hemoglobin. Hemoglobin A_{1_c} is the result of the post translational reaction of glucose with hemoglobin and has a glucose molecule attached to the terminal amino groups of the β-chains of human hemoglobin A_o. Interestingly, this single modification is found to alter the interaction of hemoglobin with Cu(II) so that hemoglobin A_{1_c} is more similar to horse hemoglobin than human hemoglobin with respect to its reactions with copper.

E. The oxidation of hemoglobin by copper. We have investigated the effect of pH on the oxidation and find that even though hemoglobin is generally considered less stable at low pH. The rate of Cu(II) oxidation decreases appreciably below pH 6.5. This decrease is also found to correspond to a decrease in the affinity of hemoglobin for Cu(II). A relationship between these changes and the previously reported changes in ESR spectra of Cu(II)-hemoglobin at low pH are being investigated. These results can help determine the types of amino acids involved in the Cu(II) binding and the mechanism for the electron transfer process.

Significance to Biomedical Research and the Program of the Institute: The physiological role of hemoglobin is to transport oxygen from the lungs to the cells. The efficient uptake and release of oxygen requires cooperative oxygen binding and the proper regulation of the oxygen affinity. It is also necessary to maintain the integrity of the erythrocyte and to limit the oxidation of hemoglobin in order to maintain an adequate concentration of functional hemoglobin in circulation. These studies thus help to elucidate a vital function of organisms. The aging process can involve changes in the ability of the organism to transport oxygen to certain tissues.

Proposed Course of the Project: (1) We plan to study the oxygenation of whole blood from individuals of various ages and try to correlate any observed changes with alterations in the erythrocyte composition and/or the hemoglobin molecule. (2) We plan to investigate the possible physiological significance of the reported zinc-induced increase in the oxygen affinity of hemoglobin. (3) We plan to further investigate the binding site for metal ions on hemoglobin and the mechanism whereby the bound metal ions alter functional properties of hemoglobin. (4) We plan to further study the interaction of metal ions with erythrocyte membranes and how these interactions alter the functional properties of the membrane. (5) We plan to study membrane fluidity and possible changes with age in the mobility of different membrane components.

Publications:

Rifkind, J.M.: The oxidation of (horse) hemoglobin by copper: An intermediate detected by electron spin resonance. Biochemistry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00087-06 LCMB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Mechanism of the Parental Age Effects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. L. Schneider	Medical Officer, PHS	LCMB NIA
OTHER:	D. Kram	Staff Fellow	LCMB NIA
	G. Bynum	Medical Officer, PHS	LCMB NIA

COOPERATING UNITS (if any)

University of California Livermore Laboratory

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Section on Cellular Aging and Genetics

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

With increasing parental age, there is an exponential increase in the frequency of children born with chromosomal disorders. Most studies indicate that this effect is chiefly due to maternal aging. Current research is directed at the potential role of paternal aging. The latter area is being approached by measuring the genetic complement of sperm samples obtained from members of the Baltimore Longitudinal Study.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: It has been clearly established that with increased maternal age there is a greatly increased risk of children being born with chromosomal disorders. Despite considerable speculation about the cause of this maternal age effect, research to delineate the mechanisms of this effect has been limited by the practical as well as ethical considerations of human experimentation.

Recent evidence indicates that advanced paternal age may also contribute to the increased frequency of chromosomally abnormal offspring with advanced parental age. This paternal age component can be directly evaluated by examining the chromosomal complement of human sperm samples derived from volunteer members of the Baltimore Longitudinal Study.

Methods Employed: (1) Sperm samples obtained from young and old volunteer members of the Baltimore Longitudinal Study were sent to Dr. Barton Gledhill of the University of California Lawrence Livermore Laboratories for flow microfluorometric analysis. With flow microfluorometric analysis, the DNA contents of individual sperm can be measured with great accuracy. (2) Sperm samples from longitudinal subjects were also placed on slides and stained with the appropriate fluorescent stains to detect fluorescence of the Y chromosome. These Y bodies are an indicator of the number of Y chromosomes present in a single sperm. The number of double Y bodies indicating the presence of two Y chromosomes reflects the overall incidence of chromosomal aneuploidy.

Major Findings: (1) There appears to be a significant increase in the frequency of double Y bodies as a function of paternal age. (2) Flow microfluorometric analyses indicate that this technique can be applied to examining chromosomal aneuploidy in human sperm samples. Results obtained by flow microfluorometry appear to correlate well with staining for Y bodies.

Significance to Biomedical Research and the Program of the Institute: Chromosomal disorders are extraordinarily common in man with a frequency of approximately 1 in 100 live births. This frequency is considerably higher if one considers that over one-half the spontaneous abortions that occur during pregnancy are due to chromosomal abnormalities.

The increased frequency of double Y bodies suggests that with paternal aging there may be an increase in nondisjunction for the Y chromosome. If this nondisjunction occurs for other chromosomes, such as the 21st pair, it may indicate that the father's age may play a role in the etiology of the Down syndrome.

Proposed Course: Studies of the paternal age effect will continue with flow microfluorometric and cytologic analysis of sperm samples obtained from volunteer members of the Baltimore Longitudinal Study. In addition, an attempt will be made to find a mouse model for the paternal age effects seen in humans.

Publications:

Schneider, E.L.: The Dilemma of Aging Parents: Increased Risk of Genetically Abnormal Offspring. In Behnke, J.A., Finch, C.E., and Moment, G.B. (Eds.): The Biology of Aging. New York, Plenum Publishing Corp., 1978, pp. 247-261.

Fabricant, J.D. and Schneider, E.L.: Studies of the genetic and immunologic components of the maternal age effect. Dev. Biol. 66: 337-343, 1978.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00088-07 LCMB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Mechanisms of Cellular Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. L. Schneider	Medical Officer, PHS	LCMB	NIA
OTHER:	D. Kram	Staff Fellow	LCMB	NIA
	Y. Nakanishi	Visiting Fellow	LCMB	NIA
	G. Bynum	Medical Officer, PHS	LCMB	NIA
	R. Dean	Staff Fellow	LCMB	NIA
	P. Thorne	Chief, Tech. Dev. Sect.	OC	NIA

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Section on Cellular Aging and Genetics

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.3	4.3	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our major objectives are to examine cell replication as well as cellular response to DNA damage as a function of aging, in vitro in cultured human cells and in vivo in rat and mouse cell populations. Examination of phytohemagglutinin (PHA) stimulated human peripheral lymphocytes with the BrdU-differential chromatid staining techniques demonstrated that the decline in PHA response with aging was due to both a decline in the number of cells stimulated to replicate by PHA as well as a decline in the replication rate of the stimulated cells. Investigations of the effect of aging on SCE induction resulted in the following findings: 1) SCE induction in vivo by several structurally distinct compounds was diminished with aging, 2) SCE induction was decreased in post-embryonic development complementing the decline seen in aging, 3) Impairment of immune response as measured by mitogen responsiveness correlated well with the decline in SCE induction and 4) Spontaneous SCE induction does not appear to be altered with in vitro passage (in vitro aging). In vivo sister chromatid exchange analysis was applied to the in utero detection of SCE as well as to the use of this system to assess cancer chemotherapeutic agents.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: A decline in the proliferative capacities of certain cell populations is an important feature of human aging. Because of the practical and ethical limitations of in vivo human experimentation, considerable effort has been expended to create appropriate in vitro tissue culture systems as well as in vivo animal models for examining age-related alterations in cell replication.

There has been considerable concern expressed over whether in vitro human diploid fibroblast aging reflects in vivo human aging. To examine this, we are establishing large numbers of skin fibroblast cultures from members of the Baltimore Longitudinal Study. Colony size distributions, which have been shown to be an excellent index of in vitro proliferative ability, will be performed on all of these cultures. Since a number of in vivo studies are also performed on these subjects, we will be able to compare in vitro measurements with in vivo measurements obtained from the same individuals. Although in vitro studies of cell replication are important, they are like all in vitro work restricted by the limitations of an artificial environment. It was, therefore, vital that an in vivo technique be developed to examine cell replicative abilities as a function of age. Previous in vivo studies were performed with radioactive labeling or by examining the end result of cellular proliferation. However, the advent of new cytogenetic techniques permits direct examination of cell replication in vivo as well as in vitro as a function of age without the need for radioisotopes. With these techniques, cell replication can be studied in several human and rodent cell systems.

These same BrdU-differential staining techniques can be utilized for the detection of sister chromatid exchanges (SCE) in vitro and in vivo. SCE have been demonstrated to be sensitive indicators of cellular response to DNA damage. Therefore, the effect of aging on cellular response to DNA damage can be assessed in the same cell systems utilized for analysis of cellular replication.

Methods Employed: (1) For measurement of cell replication in vivo and in vitro young and old cell populations were incubated with BrdU. At increasing time intervals, animals were sacrificed or cell cultures harvested and chromosomal preparations were made. Cells that had undergone 1, 2 and 3 cell cycles in the presence of this drug could be clearly identified by characteristic banding patterns. (2) By utilizing the BrdU-differential staining technique both in vitro and in vivo, one can measure the frequency of sister chromatid exchanges (SCE) in second replication cycle cells.

Major Findings: (1) Examination of phytohemagglutinin (PHA) stimulated human peripheral lymphocytes indicated that with aging there is a decline in the number of cells responding to PHA as well as a reduction in the replication rate of the PHA responsive cell populations. (2) Investigation of mutagen induction of SCE in skin fibroblast cultures derived from young and old human volunteers demonstrated a decrease in SCE induction at high mutagen levels in old cell populations. (3) Analysis of mutagen induction of SCE in immature and mature mice and rats revealed decreased SCE formation at high mutagen concentrations in immature animals. Thus mutagen-induced SCE levels increase with post-embryonic development, plateau during maturity and then decline with aging. (4) The frequency of baseline or spontaneous SCE was measured as a function of in vitro serial passage in IMR-90 fetal lung fibroblasts. No significant alteration in spontaneous SCE levels was observed. (5) The in vivo analysis of SCE has been extended to examine the induction of SCE in utero in mice. By infusing BrdU and the mutagens to be tested, SCE induction can be examined concurrently in both fetal and maternal tissues permitting analysis of transplacental transport of mutagens. Our results indicate that exposure to teratogens which act through DNA damage can be detected at drug concentrations well below that which are teratogenic. (6) In vivo BrdU-differential chromatid staining techniques were also applied to examine the effect of different cancer chemotherapeutic agents. Mice with transplanted Ehrlich Ascites tumors were infused with BrdU and the agent to be tested. Both normal bone marrow and Erhlich Ascites tumor cell populations were then analyzed for SCE induction and for inhibition of cellular replication kinetics. This permits the examination of differential toxicity of these compounds on normal and tumor cell populations.

Significance to Biomedical Research and the Program of the Institute: Our studies of the effect of aging on cellular replication continue to demonstrate a decline as a function of aging. One potential mechanism for this decline is altered cellular response to DNA damage. The results of our studies of SCE induction indicate that with aging there is a decline in the abilities of cell populations to form SCE. This reduction in mutagen induced SCE levels with aging is the reverse of what occurs with post-embryonic development.

The in utero examination of SCE induction permits a new approach to the study of transplacental mutagens. Similarly, the ability to examine SCE induction in tumor cells in vivo permits the rapid assessment of the effect of cancer chemotherapeutic agents on both normal and tumor cell populations in an environment simulating human tumors.

Proposed Course: Cellular replication will be studied as a function of aging in several well defined cell populations. These include T lymphocytes responding to phytohemagglutinin and hepatocytes dividing after partial hepatectomy.

The mechanisms for the decline in SCE induction with aging will be examined both in vivo and in vitro. In vitro, the effect of mutagens on the viability of young and old cell populations will be examined in an attempt to discern whether the DNA repair is impaired with cellular aging. In vivo, transplantation of young bone marrow to old animals and the reverse should permit the discrimination between the effects of an old environment and intrinsic cellular aging.

In vivo SCE studies will continue to further demonstrate the utilization of in utero SCE detection for the examination of transplacental mutagens as well as to examine the usefulness of the simultaneous examination of SCE induction in normal and tumor cell populations.

A bone cell system will be developed to examine the effect of aging on bone formation.

Studies of human skin fibroblasts derived from longitudinal subjects will be continued with the emphasis remaining on collaboration with outside laboratories.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00102-03 LCMB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Comparative Mammalian Biochemistry
I. Stability of Gene Control Mechanism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Cutler	Research Chemist	LCMB NIA
Other:	R. Dean	Staff Fellow	LCMB NIA
	K. Kator	Visiting Fellow	LCMB NIA (EOD 12/1/77)

COOPERATING UNITS (if any)
Pediatric Oncology, DCT, NCI; Department of Medicine, Yale University School of Medicine; Mayo Clinic Foundation; Department of Cell Biology, Baylor College of Medicine; Cancer Research Unit, McEachern Laboratory, University of Alberta.

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Section on Macromolecules

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.6	OTHER: 0.2

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have continued our study of the possible age-dependent relaxation of specific gene expression in tissues where the presence of these genes is not expected. These studies have now included the structural genes: α and β globin, casein and α-fetoprotein, and the endogenous C-type viruses: mouse leukemia virus (MuLV) and mouse mammary tumor virus (MMTV). Both globin and α-fetoprotein RNA appear to increase in nuclei and cytoplasm of the liver for the long-lived C57BL/6J mouse strain and globin RNA in the nuclei and cytoplasm of the brain for this strain. Of particular interest is the high fraction of globin RNA found only in the liver nuclei of the old AKR mouse strain. This mouse strain has a high incidence of leukemia. A high age-dependent increase in MuLV-RNA in thymus but not in liver and brain was also found for the AKR strain. Although MuLV-RNA was found to increase with age in the nuclei and cytoplasm of liver and brain in the long-lived C57BL/6J mouse strain, MMTV-RNA was found to increase only in liver nuclei. Globin RNA also appears to increase with age in human brain, as was previously found to occur in brain tissues of the mouse.

Project Description:

Objectives: Essentially all known structures and functions of the mammalian organism become altered with increasing chronological age. Some of these alterations are believed to be related to the well known age-dependent decline in physiological performance and the increased onset frequency of many dysfunctions and diseases. A number of investigators in the medical and gerontological sciences have undertaken a compartmentalized approach in studying these time-dependent structural and functional changes, therefore limiting their area of investigation and, hopefully, the complexity of the problem.

However, it is now becoming clear that any substantial extension in the period that general mental and physical health can be maintained requires a decrease in the rate of expression of most of the aging processes. In view of the complexity of the aging processes, the probability of accomplishing this objective appears to be extremely low unless primary aging processes exist which underlie most age-dependent alterations. The general long-term objective of our research program is to determine if such primary aging processes exist and, if so, how their rate of expression is controlled. The general experimental approach taken is an evolutionary-comparative biochemistry study of mammalian species. These species are genetically closely related to one another but have substantial differences in their innate capacities to maintain optimum mental and physical health as a function of chronological age.

Our evolutionary studies of human longevity indicate a surprisingly high rate of increased maximum lifespan potential over a relatively short time period. This suggests that primary aging processes may exist and that only a few alterations of regulatory genes may be the biological basis of modern man's exceptionally long period of good health. A remarkable similarity also has been found in the physiological, biochemical and qualitative nature of age-dependent dysfunctions and diseases of mammals in general, in spite of substantial differences in their maximum lifespan potentials. These and other findings have suggested a new concept: that special anti-aging processes exist that are common to most mammalian species and act according to their levels of activity to govern the rate of expression of a common set of primary aging processes. Through a comparative biochemical approach, we hope to identify these hypothetical primary aging processes and their related anti-aging processes. In effect, we are searching for the unique biological properties of man which can account for his exceptionally long duration of health and disease-free lifespan.

Our present work continues to investigate the evolutionary aspects of human longevity by reviewing and organizing the existing literature on the comparative aspects of physiology, biochemistry and molecular biology of primates and their age-dependent dysfunctions and diseases. Laboratory work centers on investigating the informational storage and transfer systems of the cell where primary aging and their related repair and protective processes may operate. This work is divided into three research projects, entitled: (I) Stability of Gene Control Mechanisms, (II) Physico-chemical Characterization of Chromatin, and (III) Species-dependent Levels of Genetic Repair and Protective Processes. The remainder of this report concerns Project I.

The proper function of the adult mammalian organism is dependent upon maintaining the correct differentiated state of many types of cells. Any deviation from this optimum state is likely to decrease the operational effectiveness of the cell and, if of sufficient magnitude, lead to serious loss of efficiency, resulting in general dysfunctions and disease processes at the organismic level. Such dedifferentiation processes of relaxed gene expression may play a role in the appearance of neurofibrillary tangles, senile plaques, autoimmune disease, and some types of slow virus diseases and cancers. Our present aim is to examine the age-dependent stability of the differentiated cell by determining if improper specific and general gene expression occurs in specific and organ cell populations as a function of age.

Methods Employed: The general method used to investigate improper specific gene expression is to search for the presence of RNA transcribed by this gene in cells where this gene would not normally be expected to be expressed. The RNA is detected by using a radioactive complementary DNA (cDNA) probe. The cDNA probe is obtained by isolating pure messenger RNA known to code for a specific gene product and then using this RNA as a template with reverse transcriptase enzyme to synthesize the radioactive cDNA probe. RNA is then extracted from the nuclei and/or cytoplasmic fractions of certain tissues and the presence of RNA complementary to the cDNA probe is determined by DNA·RNA hybridization techniques.

We are presently investigating the age-dependent presence of α and β globin, casein, α-fetoprotein, mouse leukemia (MuLV) and mouse mammary tumor virus (MMTV) genes in brain and liver of mouse. Both of the viruses are of the C-type and have their genomes integrated into the normal cellular DNA of all the cells of the mouse strains being used. Other genes we hope to examine in the near future are human and rat insulin and growth hormone.

Major Findings: Previous work has shown an age-dependent increase of α and β globin RNA in the nuclei and cytoplasm of liver and brain in the long-lived C57BL/6J mouse strain. Similar studies were then undertaken for casein and α-fetoprotein genes in these tissues. Very little casein RNA (near the limits of detectability) was found in liver and brain, and no increase with age was evident. The α-fetoprotein studies have so far only been on liver but, as with globin, we have found about a 2-fold increase with increasing age from about 3 to 24 months.

We have also previously reported that mouse leukemia virus (MuLV)-RNA increased with age in the nuclei and cytoplasm of liver and brain in the long-lived C57BL/6J mouse strain. These studies were extended by investigating the possible age-related derepression of the mouse mammary tumor virus (MMTV). Very little MMTV-RNA was found in liver cytoplasm of brain nuclei and cytoplasm, and no indication was found of a possible age-dependent increase. However, about a 4-fold increase in MMTV-RNA was found to occur with age in liver nuclei.

An age-dependent increase in globin RNA was previously found in mouse brain. Similar studies were conducted using human tissues obtained from different aged individuals. We found that an age-dependent increase in globin RNA also appears to occur in man in the gray matter of brain. A high value of globin RNA is seen at 7 weeks of age, reaching background values at about 19 years of age. This low value was maintained up to about 40 years of age, after which it began to increase again to about 2-fold at the age of about 60 years.

The short-lived high leukemia incidence mouse strain AKR was used to determine if the age-dependent increase in globin and MuLV-RNA found in the long-lived mouse strain C57BL/6J would be accelerated and if derepression of globin and MuLV are coupled. Lifespan of the AKR strain is about 12 months. We found that in the very young mouse (2 months of age), globin RNA was low and decreased to even lower values up to 10 months of age in thymus, liver and brain. MuLV-RNA in the thymus increased with age, as expected, to about 20-fold for nuclei and 15-fold for cytoplasm. Most importantly, we found a striking increase of MuLV-RNA in liver nuclei of about 9-fold with increasing age, but no MuLV-RNA in cytoplasm. For the nuclei or cytoplasm of brain, MuLV-RNA was low and showed little age-dependent increase.

Significance to Biomedical Research and Program of the Institute: These findings so far indicate that all structural genes do not undergo an age-dependent derepression and that, instead, this derepression is likely to be highly specific, not only to the type of structural gene involved but also to the type of tissue, cell fraction (nuclei or cytoplasmic) and strain or species of animal. In contrast to structural genes, however, an age-dependent derepression of endogenous C-type viruses related to cancer and other diseases may be universal. These results, if confirmed by more extensive studies which are underway, could lead to the identification of an important genetic alteration of cells which may underlie many of the mammalian aging processes.

Proposed Course of the Project: A more extensive and detailed study is now being undertaken to further characterize the age-dependent change in expression of α and β globin, α-fetoprotein, casein and the MuLV and MMTV C-type virus genes. This will involve other tissues and different mouse strains (such as AKR and C57BL/6J). Globin gene expression in human tissues will continue to be studied, and we hope to soon begin the study of insulin and growth hormone genes for both man and mouse. It is also of interest to determine if loss of cellular proliferation of *in vitro*-aged tissue culture cells involves a dedifferentiation process. We are therefore planning to examine globin gene expression in human cells (WI-38) as a function of passage number.

Our long-term proposed course will be to determine (1) the generality of age-dependent relaxed gene expression in terms of tissues and number of different genes effected, (2) if a correlation exists between aging rate of a species and the rate of loss of proper gene control, (3) the molecular mechanisms leading to a loss of gene control and (4) to identify the biological processes that may govern the age-dependent stability of gene expression. These latter two objectives are the projects of the second and third parts of our research program.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00103-03 LCMB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Comparative Mammalian Biochemistry
II. Characterization of Chromatin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Cutler	Research Chemist	LCMB NIA
Other:	R. Dean	Staff Fellow	LCMB NIA

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Cellular & Molecular Biology

SECTION
Section on Macromolecules

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.4	OTHER: 0.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An age-dependent increase in improper gene expression could result from epigenetic and/or mutational-like changes in the genetic apparatus of cells. This project is designed to determine if such mutational or physico-chemical alterations of chromatin occur that could account for the altered gene expression previously found. We have continued to investigate the T-3 hormone binding capacity and affinity to chromatin and whole nuclei in rat liver as a function of age. There is still an indication that an age-dependent decrease in binding capacity occurs, although considerable fluctuation is found. Little change with age in the binding constants were apparent. We have also investigated T-3 hormone binding capacity in old hypophysectomized rats. These animals have undergone an apparent reversal in a number of immunological parameters and kidney function, approaching that of a young animal. However, no change was found in the low T-3 binding capacity typical for the old non-hypophysectomized animals. However these old hypophysectomized rats were found to undergo a remarkable reversal in a number of morphological parameters of the kidney, as determined by electron microscopy. GRC/LCMB-124

Project Description:

Objectives: This project is designed to investigate whether the genetic apparatus of cells remains intact throughout the lifespan of the animal or if significant physico-chemical or mutational alterations occur that could lead to the improper function of the cell and possibly to the aging of the organism. The specific objective of these studies is to search for alterations at the chromatin level which might help explain the possible dedifferentiation processes that have been found to occur with increased age in mouse brain and liver tissues.

Methods Employed: The major genetic material of mammalian cells consists of a DNA-protein complex called chromatin. Very little, if any, DNA-DNA, DNA-protein and protein-protein complexes of a covalent nature are found in chromatin but could be caused by a number of mutagenic and/or carcinogenic agents, as well as many other natural by-products of metabolism that exist in a cell. Although the cell is likely to have repair and protective processes to lower the damage rate of chromatin, the accumulation of such damage could have far-reaching consequences to the proper function of the cell and organism.

We are developing techniques to detect the type and amount of DNA-protein complexes in chromatin as a function of age in C57BL/6J male mice. These are (1) the extractability of chromatin proteins from DNA, (2) the presence and stability of DNA-protein complexes as detected by a nitrocellulose membrane filter assay and (3) the thermal stability of native chromatin complexes. These techniques are rather crude, however, and could only be expected to detect rather large and non-specific changes.

More sensitive and specific methods are therefore being used, such as the binding capacity and affinity of T-3 hormone to chromatin. T-3 hormone is known to bind with high specificity directly to a specific acidic protein on the chromatin without first binding to a cytoplasmic receptor protein. Moreover, of additional interest to this method is the report that 6-month post-hypophysectomized rats are rejuvenated in a number of physiological parameters, which might be a result of renewed sensitivity of cells to T-3 hormone stimuation.

Major Findings: Our work has concentrated on the T-3 hormone binding assay to determine if any specific age-dependent alterations in chromatin might occur and if this change might be reversed in old hypophysectomized animals. Results indicate that about a 20-50% decrease with increasing age occurs in the total amount of T-3 bound per unit DNA in rat liver for either chromatin or whole nuclei preparations. Animals hypophysectomized at about 18 months of age and investigated at age 24 showed a similar decrease with age in T-3 binding capacity. Thus, hypophysectomy does not appear to interfere with this age-dependent change. A substantial variation is found between animals in the binding affinity of T-3 hormone, but no significant age-dependent changes are apparent.

The old hypophysectomized rats (4 months post-operative) have been reported to undergo a reversal in kidney function, approaching that for a young 6-month old rat. Collaborative studies with Dr. Johnson were undertaken to investigate the general morphology of the kidney of normal young and old rats and of old hypophysectomized rats. These studies indicate that, at the structural level, the kidneys do show age-dependent changes which are reversed to young levels in the hypophysectomized animals. The parameters measured were: glomerular diameter and amount of dense bodies detected in the podecytes, mesagial and endothelial cells.

Significance to Biomedical Research and Program of the Institute: Much of the aging process could theoretically be explained by an age-dependent alteration of the genetic apparatus of cells. It is therefore essential to determine if chromatin (the major genetic constituent of cells) is altered with age, and if so, if this alteration is reversible. In terms of the T-3 receptor protein, although less receptor appears to be available for binding in older animals, this decrease may not be reversible by hypophysectomy. On the other hand, hypophysectomy does appear to be able to reverse a number of physiological as well as morphological parameters. Determination of what types of aging are indeed reversible could prove important in developing new types of medical care for the elderly.

Proposed Course of the Project: A more detailed study is now underway to determine the specific biochemical nature of the physico-chemical alterations which have been found in chromatin. We plan on further developing our assay techniques to increase their sensitivity, to utilize a high-pressure liquid chromatographic system (HPLC) to detect and resolve some of the possible DNA-protein adducts that might exist in the chromatin of older mice and to use specific types of cell populations taken from liver and brain tissues. We also plan to further develop the T-3 hormone chromatin binding assay to increase its reliability and sensitivity. Through these efforts, we hope to determine what effects the chromatin alterations may have on the transcription activity of a cell and its effect on the ability of cells to maintain their proper differentiated state.

Publications:

Dean, R.G. and Cutler, R.G.: Absence of significant age-dependent increase of single-stranded DNA extracted from mouse liver nuclei. *Exp. Gerontol.* 13: 287-292, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00105-02 LCMB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Comparative Mammalian Biochemistry
III. Levels of Repair/Protective Processes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Cutler	Research Chemist	LCMB NIA
Other:	K. Kator	Visiting Fellow	LCMB NIA

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Section on Macromolecules

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 1.4	PROFESSIONAL: 0.7	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to determine if a correlation exists between levels of potential genetic repair and protective processes and the aging rate of the mammalian species. Work has continued on the superoxide dismutase (SOD) and guanylate cyclase specific activity studies using brain, liver and heart tissues of 2 rodent and 12 primate species. These species covered a maximum lifespan potential (MLP) range of 3.5 to 95 years. No correlation was found between the level of SOD specific activity and MLP. However, an excellent correlation was found between the ratio of SOD and specific metabolic rate with MLP. Preliminary data with catalase shows similar results. These results suggest that longer lived species have superior protection against oxygen toxicity. Measurement of guanylate cyclase (GC) specific activity for the same species indicates an inverse correlation with SOD specific activity. In view of the possible activation of GC by hydroxyl radicals, these results suggest that GC specific activity may be involved in governing the level of SOD specific activity and perhaps other intracellular free radical protective processes.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project represents a search for genetic repair and protective processes which might act to govern the time-dependent stability of gene expression, and particularly those processes which may play an important role in general maintenance of health in man. The approach used is to compare levels of potential genetic repair and protective processes with the maximum lifespan potentials of different mammalian species, with emphasis being placed on the primate species. To date we have investigated the selective degradation of abnormal proteins and the levels of superoxide dismutase, catalase, and guanylate cyclase in both primate and rodent species.

Methods Employed: SOD Assay. The inhibition of pyrogallol autoxidation by SOD was used to measure the relative specific activity of this enzyme in cytoplasmic extracts. Specificity of the assay for SOD activity is based on finding over 95% inhibition of pyrogallol autoxidation on addition of Cu-Zn bovine SOD to solutions with and without cytoplasmic extract. One cycle of freeze-and-thaw decreased SOD specific activity by about 6% for all tissues and species. No loss of SOD specific activity upon storage at -70°C was evident for liver, and loss was about 3% per year for brain and heart. Precision of the SOD assay was determined by four independent assays on the same human liver sample, which gave a mean ± SD value of 43.1 ± 2.15 units/mg protein. Similar precision was found for other tissues and species.

SOD Controls. Reducing agents and peroxidases are known to interfere with the autoxidation of pyrogallol. To test for such possible interference in our assays, we added pure Cu-Zn bovine SOD of known activity to cytoplasmic extracts of known activity from liver of man, Rhesus and tamarin. Percent difference from the predicted sum of SOD was 3.69 ± 2.87, which is within precision of the assay. Peroxidase interference was checked by addition of 0.2 uM catalase to cytoplasmic liver extracts of man, Rhesus and tamarin. An average increase of about 15% was found, with no significant differences between species. Percent recovery of SOD in supernatant fraction showed no species difference and averaged about 75%. No difference was found between perfused and non-perfused liver in mice. Body temperatures of primates are within one to two degrees Centigrade of one another, so species differences in SOD specific activity resulting from all assays made at 23°C are unlikely.

SOD Specific Activity in Human Tissues. Rodent tissues were frozen in dry ice immediately after death. For non-human primates, the average time after death before freezing was about 3 hr; however, for human the average time was 10.2 hr for liver, 9.4 hr for brain and 5.5 hr for heart. Possible loss of SOD activity during this time was evaluated by allowing a thawed tissue to remain at room temperature for various times before it was assayed. SOD specific activity was found to decrease in an exponential manner, with $t_{\frac{1}{2}}$ of 139 hr for liver, 14.2 hr for brain and 8.78 hr for heart.

Guanylate Cyclase Assay. Guanylate cyclase activity was assayed in the crude cytoplasmic supernatant fraction that was also used for the SOD assay. The assay method is based on the rate of production of cyclic GMP from GTP and is similar to that described by Liang and Sacktor of the Laboratory of Molecular Aging, GRC, NIA.

Major Findings: Superoxide dismutase (SOD) specific activity was measured in the cytoplasmic fractions of liver, brain and heart of 2 rodent and 12 primate species. These species had lifespans ranging from about 3.5 to 95 years. For a given species, liver, brain and heart had similar specific activity levels, and between species, levels ranged over 2-fold. No correlation was found in the level of SOD specific activity with lifespan. However, when SOD specific activity is divided by the organ's in vivo specific metabolic rate (calories per gram per day), an excellent linear correlation is found with lifespan. Straight lines of similar slope passing through the origin were found with correlation coefficients of 0.998 for liver, 0.993 for brain and 0.925 for heart. These correlations are particularly significant in view that, although specific metabolic rate generally decreases with increasing lifespan, there are many exceptions, such as the lemur, tamarin, gorilla and man. These results suggest that SOD = k (MCC), where MCC is the total calorie consumption over the lifespan of the species. MCC is defined as the product of specific metabolic rate (SMR) and maximum lifespan potential (MLP), or MCC = SMR·MLP. Preliminary results measuring the specific activity levels of catalase with lifespan of mammals indicate a similar correlation.

Guanylate cyclase specific activity levels in these same rodent and primate species were found to follow an inverse correlation with SOD specific activity. In view that guanylate cyclase may be activated by the hydroxyl free radical, this correlation suggests that this enzyme could play an important role in governing the level of SOD and other protective, as well as, repair processes against oxygen toxicity.

Significance to Biomedical Research and Program of the Institute: These studies support the concept that an increase in the level of activity of a common set of repair and protective processes in the mammalian species may play an important role in governing their different aging rates. It is also possible that the processes being studied could act to protect the genetic apparatus and thus be important in stabilizing the differentiated state of the cell. If MLP and health maintenance of primates are governed in part by the level of expression of a commonly held set of genetic repair and protective processes, then this may offer a basis to further enhance man's innate resistance to chemical and radiation hazards. In addition, when toxicity data derived from short-lived species such as rat is used to determine safe levels for man, the possible higher constitutive levels of genetic repair and protective processes in man may need to be considered.

Proposed Course of the Project: Superoxide dismutase, catalase and guanylate cyclase levels will be measured in additional primate species and in other tissues. We plan to extend these investigations to include glutathione peroxidase, cAMP, cGMP, adenylate cyclase and the depurination DNA repair levels in rodent and primate species. In addition, a number of enzymes not related to repair and protection will be studied as controls, such as aldolase and glucose-6-phosphate dehydrogenase.

Publications: None.

Laboratory of Molecular Aging

The Laboratory of Molecular Aging has made important programmatic advances in describing the physiological, biochemical, and biophysical bases for specific medical problems of the aging and the aged. Our research this year has again focused on the mechanisms whereby age-dependent changes in membrane function and transport perturb physiological control systems and, thus, lead to the failure to maintain homeostasis in the aged. These investigations impact on critical functions of the renal, cardiac, gastrointestinal, endocrine, and mineral metabolic systems and on oral and dental tissue health.

A novel project was initiated to investigate systematically oral and dental physiology during the aging process. The study assesses and defines the oral physiological status of participants in the Baltimore Longitudinal Study and examines in man and animal models biological factors which are likely to influence oral health. The latter include studies of salivary gland function, *i.e.*, saliva output:quantity and constituents, and its regulation with age. Initial focus is on mechanisms involved in protein secretion from the parotid, particularly the role of β-adrenergic receptors, cyclic AMP formation, and cyclic AMP-dependent protein kinase.

A decreased velocity of Ca^{2+} transport in isolated sarcoplasmic reticulum (SR) is known to be associated with a prolonged relaxation time in the aging heart. Kinetic analysis of the changes affecting Ca^{2+} transport shows that Vmax is decreased in the SR from old rat hearts implying that the turnover rate or site density is diminished with age. To test whether the site concentration in SR decreases with age, the level of a transport intermediate, which is proportional to the enzyme concentration, has been measured. The level of the intermediate is increased only slightly in the young age group as compared to the old and the difference is not significant. This suggests that the change in Vmax is due primarily to a change in the turnover rate rather than in the number of sites.

The contribution of the passive Ca^{2+} leak to net uptake has been evaluated by measuring Ca^{2+} efflux from passively loaded vesicles. Rapid and slow phases of Ca^{2+} efflux are present in young and old SR with no detectable age difference in either component. These results lend support to the concept that an active rather than passive property of the membrane is responsible for the diminished Ca^{2+} transport rates in cardiac SR.

Cyclic AMP and protein kinase treatment of isolated SR vesicles increase their Ca^{2+} transport activity by a mechanism that is postulated to mediate the inotropic response to catecholamines in the intact myocardium. In senescent myocardium the response to catecholamines is diminished compared to that of the young adult heart implying a reduced adaptation to stress with age. Investigation of this question reveals that addition of cyclic AMP and protein kinase to SR increases significantly ($p<.005$) the Ca^{2+} transport activity in both age groups. The percent stimulation above baseline activity (55% in the young as compared to 53% in the old) does not differ significantly with age. This indicates that the distal mechanism for mediating the response to catecholamines is unaltered with age. Although the level of activity after stimulation is higher in the young group than in the old, the stimulated Ca^{2+} transport activity in the aged heart can be manipulated to exceed the baseline activity in the young adult heart.

Studies in collaboration with investigators of Clinical Physiology Branch, GRC, demonstrate that the maximal rate of force development of the isolated intervesicular septa to a near threshold concentration of isoproterenol is enhanced in myocardium from hyperthyroid compared to euthyroid rats and that this enhanced contractile response is accompanied by a significant activation of cyclic AMP-dependent protein kinase in the hyperthyroid but not in the euthyroid hearts. Dibutyryl cyclic AMP abolishes the contractile difference between the hyperthyroid and euthyroid, suggesting that the near threshold-isoproterenol-induced changes may be explained by alterations in the β-receptor.

A Ca^{2+}-dependent regulator protein (calmodulin) is found in the renal cortex cytosol and proximal tubule brush border membrane. Calmodulin in the presence of Ca^{2+}, in addition to activating cyclic nucleotide phosphodiesterase, markedly affects the activity of cyclic AMP-dependent and -independent protein kinases. The nature of the effect, activation or inhibition, and magnitude of the effect vary with different protein kinases, the protein being phosphorylated, and the Ca^{2+} concentration. Myosin light chain serves as an excellent substrate for one of the renal cyclic AMP-independent protein kinases. Studies of the mode of action of ^{125}I-calmodulin indicate that a Ca^{2+}·calmodulin·protein substrate complex is formed which interacts differently with the kinase than does the substrate by itself.

Phosphate homeostasis is a critical element in mineral metabolism and profoundly influences the progression of osteoporosis and osteomalacia. Thus, studies of the transport of phosphate by the kidney and intestine and the regulation of uptake by diet, hormones, vitamin D, and genetic mutant animal models are of prime importance to gerontological research.

We have found that phosphate is taken up by renal proximal tubule brush border membrane vesicles against a concentration gradient and that the Na^+ chemical gradient provides the driving force for the uphill transport. However, in contrast to the electrogenic Na^+ gradient-dependent transport of D-glucose, the Na^+ gradient transport of phosphate is an electroneutral process. This conclusion stems from finding that the generation of a large inside negative membrane potential by a valinomycin-induced K^+ diffusion potential has no affect on phosphate transport. The Na^+ gradient-dependent uptake of phosphate is modulated by pH, the rate of uptake being higher at more alkaline pH values. The valinomycin induced K^+ diffusion potential does not alter uptakes throughout the pH range of 6.0 to 8.5. This suggests that Na^+-phosphate cotransport is electroneutral at all the pH values tested even though at pH 6.0 the predominant phosphate species is $H_2PO_4^-$ whereas at 8.5 it is HPO_4^{2-}. When the rate of phosphate uptake was measured at various pH values but keeping the concentration of HPO_4^{2-} constant, transport still shows an alkaline pH optimum. This finding precludes the possibility that the alkaline optimum is due solely to the fact that at higher pH values there is a greater concentration of the HPO_4^{2-} species in the reaction. Instead, it indicates that the carrier itself is pH dependent.

Animals (rabbits and mice) fed a low phosphate diet adapt to conserve filtered phosphate in that isolated renal brush border membrane vesicles show a doubled rate of phosphate uptake. This adaptation has a lag period of several days and is found only when measured with a Na^+ gradient. Phosphate uptake in the absence of Na^+ or in the presence of Na^+ but with $[Na^+]_o = [Na^+]_i$ show no enhanced uptake. This argues against solely an increase in the number of carriers as the mechanism for the adaptation. The increase in transport is specific for phosphate, since the uptakes of D-glucose and L-proline (cotransported electrogenically with Na^+) or L-glutamate (cotransported electroneutrally with Na^+) are unchanged. Kinetic studies indicate that the stimulation of phosphate uptake in membrane vesicles is not due to an alteration in the affinity of the carrier for phosphate. The Vmax of the system is increased. In contrast, the relationship between rate of phosphate uptake and Na^+ concentration does indicate change. Both the Vmax and the Km for Na^+ increase, when measured at 100 μM phosphate. This finding indicates that the adaptation to a low phosphate diet involves a change in the interaction of the Na^+ gradient with the phosphate carrier.

When renal cortical slices are incubated with parathyroid hormone (30 units/ml) and then the brush border membranes of the tissue isolated, the membrane vesicles show a decrease (about 10-15%) in the rate of phosphate uptake. Only the Na^+ gradient-dependent uptake of phosphate is affected. The decrement in transport is specific for phosphate as the Na^+ gradient-dependent transport of D-glucose is unaffected.

Animals on a high phosphate diet have an elevated level of circulating PTH. This prompted us to examine the effect of dietary manipulation on renal membrane PTH-sensitive adenylate cyclase. PTH-sensitive adenylate cyclase activity is 30% lower in mice on a high phosphate diet than on a low phosphate diet, being 4- and 6-fold basal activity, respectively. The diet does not affect basal, NaF-stimulated, nor calcitonin-stimulated activities. The attenuation in hormonal response is not due to a deficient coupling of PTH activation with guanine nucleotide. A kinetic study of adenylate cyclase activity at various concentrations of PTH indicates that the lower PTH-response with the high phosphate diet is the result of a change in Vmax. Half-maximum activation is about 3 units of PTH/ml reaction mixture with both diets. These results provide evidence consistent with *in vivo*desensitization of renal adenylate cyclase to PTH.

Renal cortex slices incubated with PTH markedly accumulate cyclic AMP. However, slices from mice on a high phosphate diet have a lower cyclic AMP content relative to those on a low phosphate diet, a finding in accord with a decreased PTH-stimulated adenylate cyclase in the isolated renal membrane. The increased tissue levels of cyclic AMP, induced by PTH, are correlated with increases in cyclic AMP-dependent protein kinase activity. The activation of protein kinase (increase in -cyclic AMP/+cyclic AMP ratio) is less in animals on a high phosphate diet. This observation is also consistent with down regulation.

In control animals, not treated with PTH, the concentration of cyclic AMP in the renal cortex and the state of activation of protein kinase (-cyclic AMP/ +cyclic AMP ratio) in the kidneys of animals on a low phosphate diet are the same as those on a high phosphate diet. These findings suggest that factors other than parathyroid hormone (PTH) are implicated in the regulation of phosphate uptake by dietary manipulation.

Studies in collaboration with Drs. Scriver and Tennenhouse, McGill University, reveal that brush border membranes from the X-linked vitamin D-resistant hypophosphatemic mouse have a decreased (50%) rate of phosphate transport. This loss is found only in the presence of a Na^+ gradient and is specific for phosphate. Brush border membrane vesicles from the intestine do not show this genetic defect. The biochemical lesion of this genetic disturbance is being characterized.

Studies on the mechanism of the renal transport of *myo*-inositol by brush border membrane vesicles have provided an explanation for the clinical observation of inosituria in hyperglycemia. *myo*-Inositol enters the glomerular filtrate freely and is reabsorbed by the renal tubule. Hyperglycemia leads to inosituria. Because the transport of *myo*-inositol by renal membranes is virtually unexplored, we (in collaboration with M. R. Hammerman and W. H. Daughaday, Washington University) have examined the mechanism of *myo*-inositol uptake by rabbit renal proximal tubule brush border membrane vesicles and characterized the relationship between the transports of *myo*-inositol and D-glucose. It has been found that the uptake of *myo*-inositol by brush border membranes represents transport into membrane vesicles rather than binding to the membrane and that the *myo*-inositol taken up by the membrane vesicles is not metabolized. A 100 mM Na^+ electrochemical gradient (extravesicular medium>intravesicular medium) stimulates the initial rate of *myo*-inositol uptake 20- to 60-fold. Other cation gradients are ineffective. At the peak of the "overshoot" the accumulation of *myo*-inositol is 10-times greater than the final equilibrium value. This finding indicates that the Na^+ electrochemical gradient provides the driving force to transport *myo*-inositol into the membrane vesicles against its concentration gradient. The generation of a membrane potential (inside negative) by a valinomycin-induced K^+ diffusion potential or anions with increased conductances enhances Na^+-*myo*-inositol cotransport. These observations suggest that the Na^+-*myo*-inositol cotransport system is electrogenic and, therefore, rheogenic. The Na^+ electrochemical gradient-dependent uptake of *myo*-inositol saturates at about 1 mM *myo*-inositol, with an apparent Km of 94 µM at an initial 100 mM Na^+ gradient.

D-Glucose is a competitive inhibitor of the Na^+ electrochemical gradient-dependent uptake of *myo*-inositol with an apparent K_i of 3.7 mM. High concentrations of phlorizin inhibits *myo*-inositol uptake. D-Glucose, but not L-glucose nor L-proline, elicits accelerated exchange diffusion of *myo*-inositol. *myo*-Inositol does not significantly inhibit the Na^+ gradient-dependent transport of D-glucose. These findings are interpreted to suggest that D-glucose inhibits *myo*-inositol uptake by dissipating the membrane potential and sharing the *myo*-inositol carrier. On the other hand, *myo*-inositol is not a significant substrate for the D-glucose carrier. The inhibition of *myo*-inositol transport across the brush border membrane by D-glucose may explain how hyperglycemia, with its resultant glycosuria, produces inosituria in patients with diabetes mellitus.

Continuing studies on the mechanisms of amino acid transport reveal a novel and unexpected discovery. Last year, we reported that the carrier-mediated transport of L-glutamate by renal proximal tubule brush border membranes is Na^+ gradient-dependent, stereospecific, concentrative, and electroneutral, in contrast to the electrogenic transport systems of the amino acids L-proline and L-alanine, and D-glucose. We now find that the Na^+ gradient ($[Na^+]_o > [Na^+]_i$)-dependent accumulation of L-glutamate, but not those of L-proline nor D-glucose, is stimulated several-fold by internal K^+. This effect is specific for K^+ or Rb^+. That an internal gradient of K^+ is required for stimulation of Na^+ gradient-dependent transport of L-glutamate is suggested by the observations that (1) lowering $[K^+]_i/[K^+]_o$ by increasing $[K^+]_o$ progressively decreases the level of overshoot seen for L-glutamate and (2) valinomycin and nigericin both inhibit stimulation by internal K^+. The high degree of specificity and affinity (Km) of these membrane vesicles for L-glutamate and closely related analogs in the presence of Na^+ is unchanged in the presence of an internal K^+ gradient. Only the Vmax of the carrier appears to be affected by $[K^+]_i > [K^+]_o$. This suggests that the ($[Na^+]_o > [Na^+]_i$)-dependent and ($[Na^+]_o > [Na^+]_i + [K^+]_i > [K^+]_o$)-dependent systems represent functions of the same glutamate carrier. That movement of K^+ is indeed coupled to the movement of Na^+ and glutmate is indicated by the finding that the uphill transport of glutamate can be energized by the internal K^+ gradient alone. K^+ gradient-driven accumulation of glutmate is observed only in the presence of Na^+ ($[Na^+]_o = [Na^+]_i$) and is not inhibited by ouabain. K^+ gradient-dependent transport of glutamate is not affected by manipulations that alter the membrane potential and therefore appears to be an electroneutral process as is the Na^+-gradient dependent system. In light of these observations several alternative but feasible stoichiometries for sodium, potassium, and glutamate must be considered. Moreover, if this system is to be found *in situ*, then a possible mechanism for K^+ secretion in the proximal tubule is evident, a process heretofore unreported.

A carrier-mediated, Na^+ gradient-dependent, uphill, transport system for arginine has been discovered in renal brush border membrane vesicles. Previous attempts to demonstrate such a system have been unsuccessful, although electrophysiological studies in the perfused tubule, *in situ*, (by Fromter) have predicted its presence. Successful demonstration with the membrane vesicles requires the generation of large valinomycin-induced K^+ diffusion potentials, inside negative, as a driving force. This arginine transport system is stereospecific, shared by lysine and ornithine, and inhibited by cystine, as predicted by genetic diseases involving defects in the basic amino acid transport.

Studies on the mechanism by which sulfate is transported by renal proximal tubule brush border membrane vesicles have been initiated. The imposition of a large sodium chloride gradient ($[NaCl]_o > [NaCl]_i$) results in a marked increase in the initial rate of uptake and in the transient accumulation of sulfate against its concentration gradient, with an "overshoot" similar to that seen with glucose, proline, glutamate, and phosphate. This overshoot is not observed in the absence of a Na^+ gradient. However, the initial rate of uptake of sulfate in the presence of Na^+ (no gradient) is 4 and 7 times greater than that for K^+ or Tris, respectively. Na^+ gradient-dependent uptake of sulfate is an electroneutral process. We have observed that Na^+ gradient-dependent transport of sulfate is markedly stimulated by internal permeant anion, either Cl^- or NO_3^- but not by internal impermeant anions such as glutamate or gluconate. Moreover, SITS, an inhibitor of anion exchange in the red blood cell, also inhibits the Na^+ gradient-dependent uptake of sulfate in the presence of internal Cl^-. The exact nature of this stimulation by Cl^- and inhibition by SITS requires further investigation. Internal K^+ also stimulates Na^+ gradient-dependent transport of sulfate about two-fold (regardless of internal permeant or impermeant anion) and is indeed specific for K^+ and Rb^+. The mechanism of the K^+ stimulation of sulfate transport is different from that for glutamate transport. First of all, external K^+ has no effect on internal K^+-stimulated Na^+ gradient-dependent transport of sulfate. Secondly, an internal K^+ gradient ($[K^+]_i > [K^+]_o$) is not sufficient to drive transport of sulfate in the presence of Na^+, as it is in transport of glutamate.

In collaborative studies with Drs. Paul Lietman and James Lipsky, Clinical Pharmacology Division, Johns Hopkins Univ. School of Medicine, we have examined the uptake of ^3H-gentamicin by isolated brush border membrane vesicles. Our findings suggest that the uptake of ^3H-gentamicin by brush border membrane represents binding to the membrane rather than transport via a specific carrier across the membrane into an intravesicular space. Binding is concentration-dependent; an affinity constant of 5×10^3 l/mole was found by Scatchard analysis. Bound gentamicin is displaced by spermine. These studies of the interaction of the antibiotic with the membrane may be relevant to its renal accumulation and nephrotoxicity and may suggest preventative measures.

Papain serves as a valuable tool in studies of the structural and functional organization of the renal brush border membrane. Treatment of right-side out membrane vesicles with papain leads to progressive proteolysis, resulting in the removal of about one third of the total brush border protein. Membrane-bound enzymes are differentially released. Leucine amino peptidase is completely solubilized (50% at 0.5 μg papain/mg protein), whereas trehalase and γ-glutamyl transpeptidase are remarkably resistant. Alkaline phosphatase and maltase show intermediate sensitivities. Transport functions are also differentially affected. D-Glucose and L-proline transport are not altered even at high papain concentrations suggesting that these partially digested membranes still maintain a normal Na^+ electrochemical gradient. In contrast, the Na^+-dependent uptakes of phosphate and glutamate are severely impaired (by 70-80%) suggesting a basic difference in the organization of the two electrogenic transport systems from the two electroneutral transport systems.

Two different techniques have been developed to achieve internalization of papain in the membrane vesicle. With either procedure it is clear that Na^+ gradient-dependent D-glucose uptake is now partially susceptible (∿50%) to papain proteolysis suggesting that essential components of the glucose transport system are located at the inner membrane surface of the brush border membrane vesicle. Phosphate uptake, in contrast, is now totally abolished. These findings suggest that essential papain-sensitive component(s) of the glucose transport system are located on the internal surface of the membrane whereas essential components of the phosphate transport system are found both at the external and internal membrane surfaces accessible to papain.

The specific activity of maltase, a specific "enzyme marker" for renal brush border membranes, in isolated brush border membranes is decreased about 70%

in aged dogs (11-14 yrs) relative to young adult dogs (2-4 yrs), 57 and 188 nmol/min/mg protein, respectively. An identical age decrement, 70%, is found in the homogenate of the renal cortex. These findings plus the observation that enrichment in brush border enzymes relative to the homogenate are similar, rule out the possibility that the decrease in brush border membrane activity is due to differences in homogeneities of the membrane preparations. Taking advantage of the finding that papain releases maltase from the brush border membrane, maltase was partially purified (400-fold) by papain extraction followed by a single chromatographic step column on Sepharose 4B. The solubilized partially purified enzyme also shows the age-decrement, 2565 vs 8610 for the "old" and "young" enzyme, respectively. The enzymes from the two aged groups do not differ in Km, molecular weight, and charge. However, differences are found in sensitivity to elevated temperature. At 54°C both the partially purified and membrane-bound enzyme from the old dog are more stable than the corresponding preparations from the young dog. Additionally, the "old" enzyme shows a biphasic temperature inactivation curve whereas the "young" enzyme has a linear curve. These findings suggest that the decrease in maltase activity may have resulted from conformational changes of the protein that have occurred in the "old" membrane-bound enzyme which are retained after release from the membrane environment.

The age-linked changes in the activity of enzymes involved in major catabolic pathways in heart have been investigated. This work was stimulated by the finding of a specific decrease with senescence in the activity of several enzymes involved in fatty acid and acylcarnitine oxidation in heart mitochondria and the general paucity of knowledge of the activity of the rate-determining enzymes of oxidative metabolism in tissues from senescent animals. These are the enzymes catalyzing reactions which are far from equilibrium in the cell. Measurements were made in homogenates of rat heart, for the reason that the isolation of a mitochondrial fraction may obscure age-changes, owing to the selective loss of more fragile organelles. The following activities have been measured, with the 24-month value being given as a percentage of the 6-month value, on a wet-weight basis: citrate synthase (82%); NAD-isocitrate dehydrogenase (84%); α-ketoglutarate dehydrogenase (81%); cytochrome *c* oxidase (76%); carnitine acetyltransferase (49%) and β-OHacyl-CoA dehydrogenase (73%). Thus, there is a concerted decrease in the activity of tricarboxylate cycle enzymes, which may reflect a lower tissue content of mitochondria, and a greater decrease in two enzymes involved in lipid metabolism. This latter finding is consistent with the findings reported last year on the decrease in the activities of these enzymes in isolated heart mitochondria from the 24-month rat.

The regulation of pyruvate dehydrogenase activity is of crucial importance to the interaction between carbohydrate and lipid oxidation, as this reaction commits pyruvate, derived from glycolysis, to the formation of acetyl groups and eventual complete oxidation to carbon dioxide. There is no pathway for the reformation of carbohydrate from acetyl groups, and so the pyruvate dehydrogenase reaction must be attenuated during the oxidation of fat and inhibited in starvation. In addition, it must respond to the energy-demands upon the tissue, expressed via the ratios of ATP/ADP and $NADH/NAD^+$. Control of pyruvate dehydrogenase is correspondingly complex but is effected, in part, by covalent modification of the protein. Involved is a cycle of phosphorylation and dephosphorylation, the former process giving rise to a catalytically-inactive protein. The amount of active enzyme (denoted PDH_a) reflects the resultant of the activities of a specific kinase enzyme and a phosphatase, and is sensitive to a change in the activity of either. Ca^{2+}-ions enhance the activity of the phosphatase thereby leading to a raised content of PDH_a. Preliminary experiments reported last year showed that respiring heart mitochondria respond to changes in the extramitochondrial Ca^{2+}-ion concentration in the range 5.5×10^{-8} M to 4.7×10^{-7} M with large and reversible changes in steady-state PDH_a content. This finding is of interest because these are plausible cytosolic free-Ca^{2+} concentrations and the reversibility of the activation makes it potentially of regulatory significance *in vivo*. We report now that the effect of Ca^{2+}-ions on PDH_a content is not mediated by a decrease in the mitochondrial ATP/ADP and $NADH/NAD^+$ ratios owing to the energy-demand of cation-uptake. The ratio acetylCoA/CoA, by contrast, increases from 0.64 to 1.6 as Ca^{2+}-ion concentration increases from zero to 4.7×10^{-7} M. This change cannot be a cause of the increased PDH_a content, but rather is likely to be an effect of it. It is concluded that the action of Ca^{2+}-ions is a direct effect at the level of the interconversion enzymes and does not depend upon an alteration of the mitochondrial energy-state. It may well increase flux through this major catabolic pathway in response to an elevated cytosolic Ca^{2+}-ion concentration and increased contractile activity of the heart.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00041-06 LMA

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Physiological Control Systems and Aging I
Membrane Transport Mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS: 8.6	PROFESSIONAL: 6.3	OTHER: 2.3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study of membrane transport is targeted to provide the basic scientific information needed to determine the mechanisms by which physiological control systems and homeostatic mechanisms are altered in the aged. Membrane vesicles derived from the luminal brush border segment and the antiluminal basal-lateral region of the renal tubule and intestinal epithelial cell plasma membrane are used. Topics investigated include: 1) the mechanism of the Na$^+$ gradient-dependent uptake of D-glucose; 2) the mechanisms and specificities of amino acid transport systems; 3) the mechanism and regulation of phosphate transport; 4) the mechanism of D-glucose-dependent Na$^+$ transport; 5) the mechanism of sulfate transport; 6) inositol transport, the mechanism of inosituria in hyperglycemia; 7) the uptake of aminoglycosides (gentamicin) and its relationship to nephrotoxicity; 8) molecular organization and orientation of membrane components in the membrane; 9) the isolation and characterization of the Na$^+$ gradient-dependent glucose carrier by reconstitution of synthetic membranes; and 10) changes in membrane function with age.

Project Description:

Objectives: These studies are targeted to define the biochemical and biophysical mechanisms whereby age-dependent changes in membrane transport perturb physiological control systems which lead to a failure to maintain homeostasis. The investigations impact on vital functions, including those in the kidney, brain, bone and mineral tissues, gastrointestinal and urogenital tracts. The thrust of the work is focused on questions dealing with the biology of membranes, including: (1) molecular organization; (2) role in selective vectorial transport; (3) hormonal, dietary and genetic regulation of function; (4) catalytic function; (5) turnover; and (6) failure to maintain structure, leading to cell death.

Methods Employed: Membrane vesicles derived from the luminal brush border segment and the antiluminal basal-lateral region of the renal tubule and intestinal epithelial cell plasma membrane are used as model systems.

Major Findings:

Amino Acid Transport Mechanisms — Last year, we reported that the carrier-mediated transport of L-glutamate by renal proximal tubule brush border membranes is Na^+ gradient-dependent, stereospecific, concentrative, and electroneutral, in contrast to the electrogenic transport systems of the amino acids L-proline and L-alanine, and D-glucose. We now find that the Na^+ gradient ($[Na^+]_o > [Na^+]_i$)-dependent accumulation of L-glutamate, but not those of L-proline nor D-glucose, is stimulated several-fold by internal K^+. This effect is specific for K^+ or Rb^+; corresponding salts of Li^+, choline, tetraethylammonium, and Tris do not stimulate above the level of iso-osmolar mannitol. That an internal gradient of K^+ is required for stimulation of Na^+ gradient-dependent transport of L-glutamate is suggested by the observations that (1) lowering $[K^+]_i/[K^+]_o$ by increasing $[K^+]_o$ progressively decreases the level of overshoot seen for L-glutamate and (2) valinomycin and nigericin both inhibit stimulation by internal K^+. The high degree of specificity and affinity (Km) of these membrane vesicles for L-glutamate and closely related analogs in the presence of Na^+ is unchanged in the presence of an internal K^+ gradient. Only the Vmax of the carrier appears to be affected by $[K^+]_i$.$[K^+]_o$. This suggests that the ($[Na^+]_o > [Na^+]_i$)-dependent and ($[Na^+]_o > [Na^+]_i + [K^+]_i > [K^+]_o$)-dependent systems represent functions of the same glutamate carrier. That movement of K^+ is indeed coupled to the movement of Na^+ and glutmate is indicated by the finding that the uphill transport of glutamate can be energized by the internal K^+ gradient alone. K^+ gradient-driven accumulation of glutmate is observed only in the presence of Na^+ ($[Na^+]_o = [Na^+]_i$) and is markedly reduced by the K^+ ionophore, valinomycin, and not inhibited by ouabain. K^+ gradient-dependent transport of glutamate is not affected by manipulations that alter the membrane potential and therefore appears to be an electroneutral process as is the Na^+-gradient dependent system. In light of these observations several alternative but feasible stoichiometries for sodium, potassium, and glutamate must be considered. Moreover, if this system is to be found *in situ*, then a possible mechanism for K^+ secretion in the proximal tubule is evident, a process heretofore unreported.

Glutamate transport in intestinal brush border membranes, like in the renal membrane, is Na^+ gradient-dependent and electroneutral, but, in contrast to the renal system, uptake in the intestinal membrane vesicle system is not stimulated by internal K^+.

A carrier-mediated, Na^+ gradient-dependent, uphill, transport system for arginine has been discovered in renal brush border membrane vesicles. Previous attempts to demonstrate such a system have been unsuccessful, although electrophysiological studies in the perfused tubule, *in situ*, (by Fromter) have predicted its presence. Successful demonstration with the membrane vesicles requires the generation of large valinomycin-induced K^+ diffusion potentials, inside negative, as a driving force. This arginine transport system is stereospecific, shared by lysine and ornithine, and inhibited by cystine, as predicted by genetic diseases involving defects in the basic amino acid transport.

The intestinal epithelial cell, like the renal tubular cell, is characterized by a plasma membrane that is differentiated into two distinct segments, the luminal brush border and the contraluminal basal-lateral membrane, and in both cells transepithelial transport of amino acids is a vectorial process. We have isolated and characterized both segments of the plasma membrane of the rabbit intestine and have used these preparations to examine the specificities of the various amino acid transport systems. By competition experiments, five classes of amino acids are identified: neutral (L-alanine), acidic (L-glutamate); basic (L-arginine); imino acids (L-proline); and β-amino acids (β-alanine). In the intestinal brush border membrane vesicles, Na^+ gradient-dependent transport systems have been found for the neutral, acidic, basic, and imino acids. No evidence has been obtained for a carrier-mediated uptake of the β-amino acids in the intestine, in contrast to the Na^+ gradient-dependent transport of β-alanine and taurine in renal proximal tubule brush border membrane vesicles (reported last year). In the basal-lateral membrane vesicles, the transports of L-glutamate and L-alanine are Na^+ gradient-dependent, but those of L-proline and L-arginine are independent of Na^+. Again, no evidence has been obtained for a carrier-mediated β-alanine uptake system. These findings are consistent with the hypothesis that the transcellular "active" transports of amino acids consist of a Na^+ electrogradient-dependent uphill influx at the luminar brush border membrane and a Na^+-independent or downhill efflux at the basal-lateral membrane of the intestinal epithelial cell.

In the intestinal brush border membrane, transport of labeled L-glutamate is stereospecific for the L-isomer, and is inhibited strongly only by other acidic amino acids, *i.e.*, L-glutamate and L-aspartate. Other amino acids show no or little effect. The transport of labeled L-alanine is stereospecific, strongly inhibited by other neutral amino acids, moderately inhibited by imino acids and glycine, but slightly inhibited, if at all, by acidic, basic, and β-amino acids. Uptake of L-arginine is stereospecific for the L-isomer and inhibited strongly by other basic amino acids and moderately by the neutral amino acids. The L-proline carrier is stereospecific and shared by other imino acids. Uptake of L-proline is inhibited by L-phenylalanine but not by other neutral amino acids. In the intestinal basal-lateral membrane, similar multiple and highly class specific amino acid carriers have been identified.

Sugar Transport Mechanisms — Studies on the mechanism of the Na^+-D-glucose cotransport system in renal brush border membrane vesicles have progressed. It has been found that the stoichiometry of the Na^+-D-glucose cotransport appears to vary from 0.2 to 1.0 as the Na^+ concentration is increased from 1 to 20 mM. One possible explanation of this variable ratio is the presence of a low-affinity glucose transport system in the absence of Na^+. This transport system has been found to be stereospecific, inhibited by relatively high concentrations of phlorizin, and membrane potential dependent. These findings tend to suggest that the Na^+-independent sugar transport system is the same system as the Na^+-dependent cotransport system but operating in the absence of Na^+ and, therefore, support the concept that Na^+ changes the Km of D-glucose transport.

myo-Inositol Transport, an Explanation for Inosituria in Hyperglycemia — *myo*-Inositol enters the glomerular filtrate freely and is reabsorbed by the renal tubule. Hyperglycemia leads to inosituria. Because the transport of *myo*-inositol by renal membranes is virtually unexplored, we (in collaboration with M. R. Hammerman and W. H. Daughaday, Washington University, St. Louis, MO) have examined the mechanism of *myo*-inositol uptake by rabbit renal proximal tubule brush border membrane vesicles and characterized the relationship between the transports of *myo*-inositol and D-glucose. It has been found that: (a) the equilibrium uptake of *myo*-inositol decreases with increasing medium osmolarity; (b) preincubation of the membranes with *myo*-inositol increases the uptake of *myo*-inositol; (c) the uptake of a given concentration of *myo*-inositol as well as those of D-glucose and L-amino acids, at equilibrium, are approximately the same; and (d) a Na^+ electrochemical gradient evokes an "overshoot" of *myo*-inositol uptake. These findings indicate that the uptake of *myo*-inositol by renal brush border membranes represents transport into membrane vesicles rather than binding to the membrane. The *myo*-inositol taken up by the membrane vesicles has been shown not to be metabolized.

A 100 mM Na^+ electrochemical gradient (extravesicular medium>intravesicular medium) stimulates the initial rate of *myo*-inositol uptake 20- to 60-fold. Other cation gradients are ineffective. At the peak of the "overshoot" the accumulation of *myo*-inositol is 10-times greater than the final equilibrium value. This finding indicates that the Na^+ electrochemical gradient provides the driving force to transport *myo*-inositol into the membrane vesicles against its concentration gradient. The generation of a membrane potential (inside negative) by a valinomycin-induced K^+ diffusion potential or anions with increased conductances enhances Na^+-*myo*-inositol cotransport. These observations suggest that the Na^+-*myo*-inositol cotransport system is electrogenic and, therefore, rheogenic. The Na^+ electrochemical gradient-dependent uptake of *myo*-inositol saturates at about 1 mM *myo*-inositol, with an apparent Km of 94 μM at an initial 100 mM Na^+ gradient.

D-Glucose is a competitive inhibitor of the Na^+ electrochemical gradient-dependent uptake of *myo*-inositol with an apparent K_i of 3.7 mM. High concentrations of phlorizin inhibits *myo*-inositol uptake. D-Glucose, but not L-glucose nor L-proline, elicits accelerated exchange diffusion of *myo*-inositol. *myo*-Inositol does not significantly inhibit the Na^+ gradient-dependent transport of D-glucose. These findings are interpreted to suggest that D-glucose inhibits *myo*-inositol uptake by dissipating the membrane potential and sharing the *myo*-inositol carrier. On the other hand, *myo*-inositol is not a significant substrate for the D-glucose carrier. The inhibition of *myo*-inositol transport across the brush border membrane by D-glucose may explain how hyperglycemia, with its resultant glycosuria, produces inosituria in patients with diabetes mellitus.

Phosphate Transport and Its Regulation — Last year we initiated a study of the renal transport of phosphate as part of an overall project on phosphate homeostasis and reported that renal proximal tubule brush border membrane vesicles have a carrier-mediated, Na^+ gradient-dependent phosphate transport system. We now find that phosphate is taken up by the vesicles against a concentration gradient and that the Na^+ chemical gradient provides the driving force for the uphill transport. However, in contrast to the electrogenic Na^+ gradient-dependent transport of D-glucose, the Na^+ gradient transport of phosphate is an electroneutral process. This conclusion stems from finding that the generation of a large inside negative membrane potential by a valinomycin-induced K^+ diffusion potential has no affect on phosphate transport. The Na^+ gradient-dependent uptake of phosphate is modulated by pH, the rate of uptake being higher at more alkaline pH values. The valinomycin induced K^+ diffusion potential does not alter uptakes throughout the pH range of 6.0 to 8.5. This suggests that Na^+-phosphate cotransport is electroneutral at all the pH values tested even though at pH 6.0 the predominant phosphate species is $H_2PO_4^-$ whereas at 8.5 it is HPO_4^{2-}. When the rate of phosphate uptake was measured at various pH values but keeping the concentration of HPO_4^{2-} constant, transport still shows an alkaline pH optimum. This finding precludes the possibility that the alkaline optimum is due solely to the fact that at higher pH values there is a greater concentration of the HPO_4^{2-} species in the reaction. Instead, it indicates that the carrier itself is pH dependent.

Studies of the regulation of renal phosphate transport have focused on the role of dietary intake of phosphate, modulation by parathyroid hormone, and genetic control using the X-linked hypophosphatemic mouse as a homologous model.

Animals (rabbits and mice) fed a low phosphate diet adapt to conserve filtered phosphate in that isolated renal brush border membrane vesicles show a doubled rate of phosphate uptake. This adaptation has a lag period of several days and is found only in the presence of a Na^+ gradient. Phosphate uptake in the absence of Na^+ or in the presence of Na^+ but with $[Na^+]_o = [Na^+]_i$ show no enhanced uptake. This argues against solely an increase in the number of carriers as the mechanism for the adaptation. The increase in transport is specific for phosphate, since the uptakes of D-glucose and L-proline (cotransported electrogenically with Na^+) or L-glutamate (cotransported electroneutrally with Na^+) are unchanged. Kinetic studies indicate that the stimulation of phosphate uptake in membrane vesicles is not due to an alteration in the affinity of the carrier for phosphate. The Vmax of the system is increased. In contrast, the relationship between rate of phosphate uptake and Na^+ concentration does indicate change. Both the Vmax and the Km for Na^+ increase, when measured at 100 μM phosphate. This finding indicates that the adaptation to a low phosphate diet involves a change in the interaction of the Na^+ gradient with the phosphate carrier.

When renal cortical slices are incubated with parathyroid hormone (30 units/ml) and then the brush border membranes of the tissue isolated, the membrane vesicles show a decrease (about 10-15%) in the rate of phosphate uptake. Only the Na^+ gradient-dependent uptake of phosphate is affected. The decrement in transport is specific for phosphate as the Na^+ gradient-dependent transport of D-glucose is unaffected. The mechanism of this hormonal action is being examined further.

Studies in collaboration with Drs. Scriver and Tennenhouse reveal that brush border membranes from the X-linked hypophosphatemic mouse have a decreased (50%) rate of phosphate transport. This loss is found only in the presence of a Na^+ gradient and is specific for phosphate. Brush border membrane vesicles from the intestine do not show this genetic defect. The biochemical lesion of this genetic disturbance is being characterized.

Sulfate Transport Mechanism — Studies on the mechanism by which sulfate is transported by renal proximal tubule brush border membrane vesicles have been initiated. The imposition of a large sodium chloride gradient ($[NaCl]_o > [NaCl]_i$) results in a marked increase in the initial rate of uptake and in the transient accumulation of sulfate against its concentration gradient, with an "overshoot" similar to that seen with glucose, proline, glutamate, and phosphate. This overshoot is not observed in the absence of a Na^+ gradient ($[NaCl]_i = [NaCl]_o$). However, the initial rate of uptake of sulfate in the presence of Na^+ (no gradient) is 4 and 7 times greater than that for K^+ or Tris, respectively. Na^+ gradient-dependent uptake of sulfate appears to be an electroneutral process by the following criteria: (1) sulfate transport is not affected appreciably by impermeant or permeant external anions, and (2) sulfate transport is not affected by valinomycin-induced membrane potentials, either interior negative or interior positive.

We have observed that Na^+ gradient-dependent transport of sulfate is markedly stimulated by internal permeant anion, either Cl^- or NO_3^- but not by internal impermeant anions such as glutamate or gluconate. Moreover, SITS, an inhibitor of anion exchange in the red blood cell, also inhibits the Na^+ gradient-dependent uptake of sulfate in the presence of internal Cl^-. The exact nature of this stimulation by Cl^- and inhibition by SITS requires further investigation. Internal K^+ also stimulates Na^+ gradient-dependent transport of sulfate about two-fold (regardless of internal permeant or impermeant anion) and is indeed specific for K^+ and Rb^+ but not for Tris, Li^+, nor choline. The mechanism of the K^+ stimulation of sulfate transport is different from that for glutamate transport. First of all, external K^+ has no effect on internal K^+-stimulated Na^+ gradient-dependent transport of sulfate. Secondly, an internal K^+ gradient ($[K^+]_i > [K^+]_o$) is not sufficient to drive transport of sulfate in the presence of Na^+, as it is in transport of glutamate.

Renal Uptake of Gentamicin — We, in collaborative studies with Drs. Paul Lietman and James Lipsky, Clinical Pharmacology Division, Johns Hopkins Univ. School of Medicine, examined the uptake of ^{3}H-gentamicin by isolated brush border membrane vesicles by the Millipore filtration technique. ^{3}H-Gentamicin is rapidly taken up, greater than 75% within 10 min, at 20°, pH 7.5 and with approximately 100 μg membrane protein. Uptake is largely independent of the medium osmolarity, *i.e.*, intravesicular volume. Preloading with unlabeled gentamicin inhibits the uptake of ^{3}H-gentamicin, thus there is no evidence for accelerated exchange diffusion. Generation of a K^+ diffusion potential, in the presence of valinomycin (inside negative), does not enhance uptake of ^{3}H-gentamicin. A Na^+ electrochemical gradient (outside>inside) inhibits uptake. These findings suggest that the uptake of ^{3}H-gentamicin by brush border membrane represents binding to the membrane rather than transport via a specific carrier across the membrane into an intravesicular space. Binding is concentration-dependent; an affinity constant of 5×10^3 l/mole was found by Scatchard analysis. This interaction of the antibiotic with the membrane may be relevant to its renal accumulation and nephrotoxicity.

Molecular Organization and Orientation of Membrane Components in the Brush Border Membranes — Papain serves as a valuable tool in studies of the structural and functional organization of the renal brush border membrane. Treatment of right-side out membrane vesicles with papain 0.1-35 μg/mg protein for 5 min at 37° leads to progressive proteolysis, resulting in the removal of about one third of the total brush border protein. Membrane-bound enzymes are differentially released. Leucine amino peptidase is completely solubilized (50% at 0.5 μg papain/mg protein), whereas trehalase and γ-glutamyl transpeptidase are remarkably resistant. Alkaline phosphatase and maltase show intermediate sensitivities. Transport functions are also differentially affected. D-Glucose and L-proline transport are not altered even at high papain concentrations suggesting that these partially digested membranes still maintain a normal Na^+ electrochemical gradient. In contrast, the Na^+-dependent uptakes of phosphate and glutamate are severely impaired (by 70-80%) suggesting a basic difference in the organization of the two electrogenic transport systems from the two electroneutral transport systems.

Two different techniques have been developed to achieve internalization of papain in the membrane vesicle. With either procedure it is clear that Na^+ gradient-dependent D-glucose uptake is now partially susceptible (∿50%) to papain proteolysis suggesting that essential components of the glucose transport system are located at the inner membrane surface of the brush border membrane vesicle. Phosphate uptake, in contrast, is now totally abolished. These findings suggest that essential component(s) of the glucose transport system are located on the internal surface of the membrane whereas essential components of the phosphate transport system are found both at the external and internal membrane surfaces accessible to papain.

The effect of internal proteolytic digestion on the enzyme activities of the brush border membrane reveals that the activity sites of some enzymes (*e.g.*, trehalase) are buried in the membrane in such a way that makes it totally inaccessible to papain from both the external and the internal surfaces, while others (*e.g.*, LAP) may be strictly external.

Detergent (Triton X-100) extraction of the renal brush border membranes results in the release of two proteins having trehalase activity. The two activities can be separated by sucrose density gradient centrifugation and DEAE-Sephadex column chromatography. Km values for trehalose and Ki values for Tris, $HgCl_2$, phlorizin and phloretin are slightly different for the two forms. However, the stability of one activity towards freezing is remarkably different from the stability of the other. Kinetic studies suggest the presence of two distinct inhibitor-binding sites apparently competitive with substrate. The true function of trehalase in the renal membrane is unknown at present and the activity of the enzyme decreases remarkably with aging (reported previously).

Changes in Membrane Function with Age — The specific activity of maltase, a specific "enzyme marker" for renal brush border membranes, in isolated brush border membranes is decreased about 70% in aged dogs (11-14 yrs) relative to young adult dogs (24 yrs), 57 and 188 nmol/min/mg protien, respectively. An identical age decrement, 70%, is found in the homogenate of the renal cortex. These findings plus the observation that enrichment in brush border enzymes relative to the homogenate are similar, rule out the possibility that the decrease in brush border membrane activity is due to differences in homogeneities of the membrane preparations. Taking advantage of the finding that papain releases maltase from the brush border membrane, maltase was readily partially purified (400-fold) by papain extraction followed by a single chromatographic step column on Sepharose 4B. The solubilized partially purified enzyme also shows the age-decrement, 2565 vs 8610 for the "old" and "young" enzyme, respectively. The enzymes from the two aged groups do not differ in Km, molecular weight, and charge. However, differences are found in sensitivity to elevated temperature. At 54°C both the partially purified and membrane-bound enzyme from the old dog are more stable than the corresponding preparations from the young dog. Additionally, the "old" enzyme shows a biphasic temperature inactivation curve whereas the "young" enzyme has a linear curve. These findings suggest that the decrease in maltase activity may have resulted from conformational changes of the protein that have occurred in the "old" membrane-bound enzyme which are retained after release from the membrane environment.

Significance to Biomedical Research and to the Program of the Institute: These studies, using renal plasma membrane vesicles as model membranes to investigate transport processes, describe mechanisms whereby age-dependent perturbation in physiological control systems may lead to the inability of the aged organism to maintain homeostasis. This fundamental information is needed for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities.

Proposed Course of the Project: Studies on the mechanisms of transport will: (1) further probe the mechanisms and stoichiometries of Na^+-solute cotransport systems, (2) examine the mechanism of amino acid transport in renal membrane vesicles, (3) determine the role of K^+ in amino acid transport, (4) describe the mechanism of phosphate transport and its regulation by diet, hormones, vitamin D, and genetic models, (5) examine the mechanism of sulfate transport, (6) examine Na^+-H^+ exchange systems as a mechanism of acid-base and salt-water balance, (7) explore the recent technique of membrane resolution-reconstitution to define transport systems in terms of molecular organization of the membrane, and (8) continue to apply these fundamental studies to transport systems in the kidney of the aged dog.

Publications:

Beck, J. C. and Sacktor, B.: The sodium-electrochemical potential-mediated uphill transport of D-glucose in renal brush border membrane vesicles. J. Biol. Chem. 253: 5531-5535, 1978.

Beck, J. C. and Sacktor, B.: Membrane potential-sensitive fluorescence changes during Na^+-dependent D-glucose transport in renal brush border membrane vesicles. J. Biol. Chem. 253: 7158-7162, 1978.

Hilden, S. A. and Sacktor, B.: Preservation of renal brush border membrane transport function by storage in glycerol. Kidney Int. 14: 279-282, 1978.

Hilden, S. A. and Sacktor, B.: D-Glucose-dependent sodium transport in renal brush border membrane vesicles. J. Biol. Chem., in press.

Sacktor, B.: Electrogenic and electroneutral Na^+ gradient-dependent transport systems in the renal brush border membrane vesicle. In Bronner, F. and Kleinzeller, A. (Eds.): Current Topics in Membranes and Transport, Volume 13, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00042-06 LMA

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TITLE OF PROJECT (80 characters or less)
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Mechanisms of Hormonal Regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study of hormonal regulation is targeted to define the biochemical mechanisms whereby age-dependent changes perturb physiological control systems and, thus, lead to a failure to maintain homeostasis in the aged. Investigations are focused on the biochemical interactions of hormones, which are mediated via cyclic AMP and cyclic GMP. Topics investigated include: 1) hormone receptors in membranes; 2) adenylate cyclase and guanylate cyclase activities; 3) phosphorylation of membrane proteins by cyclic nucleotide-dependent and -independent protein kinases; 4) regulation of protein phosphorylation by Ca^{2+} and Ca^{2+}-dependent regulatory protein (Calmodulin).

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Project Description:

Objectives: These studies are targeted to define the biochemical mechanisms whereby age-dependent changes in hormonal regulation perturb physiological control systems and, thus, lead to a failure to maintain homeostasis in the aged. This new information is needed as a base for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities. Investigations are focused on the biochemical interactions of hormones, mediated via cyclic AMP and cyclic GMP. The research has impact on the mechanism underlying age-related changes in renal, neural, cardiac and smooth muscle function.

Methods Employed: Membrane and cytosol preparations derived from kidney, brain and heart were used.

Major Findings:

Regulation of Phosphate Homeostasis — Phosphate homeostasis is regulated, in part, by hormones whose mode of action are mediated via cyclic AMP. Thus, studies of plasma membrane-bound hormone receptors, enzymes that control tissue levels of the cyclic nucleotide, *e.g.*, adenylate cyclase and phosphodiesterase, and enzymes whose activities are regulated by cyclic AMP, *e.g.*, protein kinases, are of great significance.

We have shown that brush border membrane vesicles from animals on a low phosphate diet (0.07%) have a two-fold, or more, increased rate of phosphate transport compared to those on a high phosphate diet (1.0%). It is also known that animals on a high phosphate diet have an elevated level of circulating PTH. This prompted us to examine the effect of dietary manipulation on renal membrane PTH-sensitive adenylate cyclase. PTH-sensitive adenylate cyclase activity is 30% lower in mice on a high phosphate diet than on a low phosphate diet, being 4- and 6-fold basal activity, respectively. The diet does not affect basal, NaF-stimulated, nor calcitonin-stimulated activities. The attenuation in hormonal response is not due to a deficient coupling of PTH activation with guanine nucleotide, since the lower PTH-stimulated adenylate cyclase from animals on the high phosphate diet is still found when enzyme activity is measured in the presence of GTP. A kinetic study of adenylate cyclase activity at various concentrations of PTH indicates that the lower PTH-response with the high phosphate diet is the result of a change in Vmax. Half-maximum activation is about 3 units of PTH/ml reaction mixture with both diets. These results provide evidence for desensitization of renal adenylate cyclase to PTH.

Renal cortex slices incubated with PTH markedly accumulate cyclic AMP. However, slices from mice on a high phosphate diet have a lower cyclic AMP content relative to those on a low phosphate diet, a finding in accord with a decreased PTH-stimulated adenylate cyclase in the isolated renal membrane. The increased tissue levels of cyclic AMP, induced by PTH, are correlated with increases in cyclic AMP-dependent protein kinase activity. The activation of protein kinase (increase in -cyclic AMP/+cyclic AMP ratio) is less in animals on a high phosphate diet. This observation is also consistent with down regulation.

In control animals, not treated with PTH, the concentration of cyclic AMP in the renal cortex and the state of activation of protein kinase (-cyclic AMP/ +cyclic AMP ratio) in the kidneys of animals on a low phosphate diet are the same as those on a high phosphate diet.

These findings suggest that factors other than parathyroid hormone (PTH) are implicated in the regulation of phosphate uptake by dietary manipulation.

Characterization of Renal Cortex Cytosolic and Brush Border Membrane Cyclic AMP-dependent Protein Kinases — We previously reported the presence of multiple cyclic AMP-dependent and -independent protein kinases in the renal cortex. Four cyclic AMP-dependent enzymes have been separated, two are localized in the cytosol and two are found in the brush border membrane. Each enzyme has been further characterized with respect to activation by various cyclic nucleotides, affinities for ATP and histone, and activity with GTP. All four kinases show a similar order of potency for activation, with cyclic AMP>cyclic IMP>cyclic GMP, and cyclic AMP>cyclic GMP by two orders of magnitude. The cytosolic kinases have been differentiated into Type I and Type II enzymes. The Type I kinase requires 3-times more cyclic AMP for half-maximal activation. The cytosolic Type II kinase and the two brush border Type II-like kinases have identical Ka values (8.5×10^{-9} M). The Km values for histone VII and ATP are approximately the same for the four enzymes, 0.41-0.64 mg/ml and 14-19 μM, respectively. None of the kinases show significant activity with GTP as substrate.

Calmodulin (Calcium-Dependent Regulator)-Protein Kinase Interactions — The effect of Ca^{2+} + calmodulin on protein kinase activity is specific for different kinases and protein substrates. The catalytic subunit of the rabbit renal cortex or beef heart cyclic AMP-dependent protein kinase is inhibited by Ca^{2+} + calmodulin. Inhibition requires the presence of both Ca^{2+} and the regulator protein. A Ca^{2+} concentration-inhibition study indicates an I_{50} of $4\text{-}5 \times 10^{-7}$ M free Ca^{2+}. Inhibition is found when the protein substrate is histone, but not seen when the substrate is protamine nor pyruvate kinase, the latter being a "physiological" C-subunit substrate. Inhibition of histone VII phosphorylation is independent of the concentration of C-subunit over a 30-fold range.

In contrast to the inhibition of C-subunit kinase activity, Ca^{2+} + calmodulin markedly stimulates (6- to 7-fold) a cyclic AMP-independent protein kinase, designated previously as the P-II enzyme, and which is found in the renal brush border membrane and in the renal cortex cytosol. Additional experimentation of the Ca^{2+} + calmodulin interaction with the P-II protein kinase shows: (1) The Ca^{2+} concentration-response relationship, with histone IIA as protein substrate, is biphasic. The peak stimulation is at 1.3 μM free Ca^{2+} but at higher concentrations of Ca^{2+} (150 μM) Ca^{2+} becomes inhibitory. (2) A stoichiometry of calmodulin:histone of 1:1 on a protein basis gives maximal stimulation. (3) Ca^{2+} + calmodulin activation is seen with histones IIA, VI, and VIII-S.

Inhibition is found with histone V. Protamine, and histones III-S and VII show little or no Ca^{2+} specific affect. (4) The inhibition of histone V phosphorylation has a K_I for Ca^{2+} of 10^{-6} M. (5) The Ca^{2+} + calmodulin response is linear over a 20-fold range of protein kinase concentration. (6) There is no lag in the response time to Ca^{2+} + calmodulin when the reaction is initiated by regulator protein or enzyme. (7) Myosin light chain serves as an excellent "physiological" substrate for the P-II enzyme activated by Ca^{2+} + calmodulin. With this physiological protein substrate high concentrations of Ca^{2+} are not inhibitory, as is the case with histone IIA. Also, in contrast to histone, a ratio of calmodulin:myosin light chain of 0.2 gives maximal response.

Purified calmodulin prepared from beef brain and iodinated with ^{125}I has been used to study the interactions of the Ca^{2+} regulatory protein with histones. Since both inhibitory and stimulatory effects on protein kinase activities have been found and both effects are dependent on the calmodulin:histone ratio, it is feasible that the results may be explained by a calmodulin-induced change in the protein substrate rather than a direct effect on the kinases. The question has been examined by determining elution profiles of ^{125}I-calmodulin on a Sephadex G-100 column, ± Ca^{2+} and ± histones. The following results have been obtained: (1) Histones V, VI, and VII induce a Ca^{2+}-dependent shift in the elution of ^{125}I-calmodulin to a higher molecular weight form. This is consistent with the finding that Ca^{2+} + calmodulin effect the phosphorylation of these histones. (2) Histone II-A induces the shift in the presence or absence of Ca^{2+}. This observation is in accord with the Ca^{2+}-independent stimulation of histone II-A phosphorylation by calmodulin. (3) Histone III-S does not affect the elution pattern of ^{125}I-calmodulin, ± Ca^{2+}, in agreement with the finding that phosphorylation of histone III-S is not influenced by calmodulin. (4) In control experiments, it has been found that histones ± Ca^{2+} do not alter the elution pattern of "non-specific" proteins, *i.e.*, ferritin and cytochrome c and that Ca^{2+} does not affect the elution patterns of histones, in the absence of calmodulin. In summary, these results suggest the formation of a Ca^{2+}·calmodulin·histone complex that behaves differently as a substrate for protein kinase than does histone by itself.

Identification of Endogenous Proteins Phosphorylated by Ca^{2+} + Calmodulin-dependent Protein Kinase — Using the technique of slab gel electrophoresis and radioautography of the phosphorylated protein bands, a Ca^{2+} stimulated phosphorylation of brush border membranes protein has been demonstrated. Added exogenous calmodulin has no marked effect. This suggests the presence of calmodulin as a membrane component, and this has been confirmed by a direct assay for calmodulin by stimulation of a specific calmodulin-dependent cyclic nucleotide phosphodiesterase.

β-Adrenergic-induced Alterations in Cyclic AMP-dependent Protein Kinase Activity in the Hyperthyroid Heart — In studies in collaboration with investigators of the Clinical Physiological Branch, GRC, we have demonstrated that the maximal rate of force development (dF/dt) of the isolated interventricular septa to a near threshold concentration of isoproterenol (10^{-9} M) is enhanced in myocardium from hyperthyroid (128 ± 4% control) compared to euthyroid rats

(105 ± 2%, $P<.01$). This enhanced contractile response is accompanied by a significant activation of cAMP-dependent protein kinase (.159 ± .008 to .218 ± .019, $P<.005$), while no significant change from baseline occurs in euthyroid septa (.152 ± .007 to .179 ± .012, NS). Dibutyryl cAMP (10^{-4} M) abolishes this contractile difference (126.5 ± 2.5% and 122.0 ± 9.2% in hyperthyroid and euthyroid, respectively), suggesting that these near threshold changes can be explained by alterations in the β-receptor. However, the maximum contractile response to isoproterenol (5×10^{-7} M) is decreased in hyperthyroid myocardium (192 ± 13%) compared to euthyroid (291 ± 37%), $P<.05$). Both the degree of protein kinase activation (.356 ± .017 and .344 ± .013) and the maximal contractile response to Ca^{2+} (335 ± 15% and 340 ± 12% in hyperthyroid and euthyroid, respectively) are similar, suggesting that the mechanism of the diminished maximum response is distal to protein kinase activation but not a function of an altered Ca^{2+}-troponin interaction. The diminished dF/dt response in the hyperthyroid hearts is accompanied by significantly lesser shortening of the contraction duration (85.6 ± 2.1% control in hyperthyroid vs 66 ± 2.8% control in euthyroid, $P<.001$). In addition, there is a significantly diminished increase in Ca^{2+} accumulation in response to cAMP protein kinase in microsomal preparations isolated from hyperthyroid compared to euthyroid hearts. This reduction may explain the diminished effect of isoproterenol on the shortening of contraction duration in hyperthyroid compared to the euthyroid myocardium, and may explain, at least in part, the diminished maximal response to isoproterenol in dF/dt.

Significance to Biomedical Research and to the Program of the Institute: These studies define the mechanisms whereby age-dependent perturbation in physiological control systems may lead to the inability of the aged organism to maintain homeostasis. This fundamental information is needed for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities.

Proposed Course of the Project: Studies on mechanisms of hormone regulation will focus on: (1) the mechanism for regulation of phosphate transport by diet, hormones, and vitamin D; (2) the regulation of adenylate cyclase activity by hormones; (3) the regulation of guanylate cyclase activity by catecholamines and regulatory protein factors; (4) the characterization and regulation of protein kinase activity; and (5) the mechanism of the reduced catecholamine responsiveness in the aged myocardium.

Publications:

Filburn, C., Colpo, F. and Sacktor, B.: Mechanism of phenothiazine inhibition of Ca^{2+}-dependent guanosine 3',5'-(cyclic) monophosphate phosphodiesterase of brain. Mol. Pharmacol. 15: 257-262, 1979.

Liang, C. T., Takenawa, T. and Sacktor, B.: Potentiation by GTP of hormone-responsive adenylate cyclase in renal cortex plasma membrane preparations. Biochem. Biophys. Res. Commun. 85: 1440-1445, 1978.

Takenawa, T. and Sacktor, B.: Tissue-specific effects of divalent cations and activators on soluble guanylate cyclase. Biochim. Biophys. Acta 566: 371-384, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00043-06 LMA

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Physiological Control Systems and Aging III
Regulation of Intermediary Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Sacktor	Chief, Lab. Molec. Aging	LMA NIA
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COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Molecular Aging

SECTION

Intermediary Metabolism Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.4	2.2	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies define the biochemical mechanisms whereby age-dependent changes in regulation of intermediary metabolism perturb physiological control systems and, thus, lead to a failure to maintain homeostasis in the aged. Investigations include: 1) regulation of carbohydrate and fat oxidation; 2) mechanism of control of pyruvate dehydrogenase activity, 3) loci of the deficits in mitochondrial oxidations in aged animals; 4) the role of Ca^{2+} in regulating the activity of bioenergetic reactions.

Project Description:

Objectives: These studies are designed to define the biochemical mechanisms whereby age-dependent changes in regulation of intermediary metabolism perturb physiological control systems and, thus, lead to a failure to maintain homeostasis in the aged. The thrust of the work is focused on mitochondrial metabolism in the aged, including regulation of the interaction of carbohydrate and fatty acid oxidative processes and control of the tricarboxylate cycle, the major energy-yielding pathway in most tissues. This research has impact on the mechanism underlying age-related changes in cardiac, hepatic and renal function as well a metabolic homeostasis.

Methods Employed: As model systems, mitochondrial preparations from various tissues and partially purified enzymes are used. Appropriate biochemical and enzymatic assays and procedures are employed.

Major Findings:

Regulation of Pyruvate Dehydrogenase Activity - The regulation of pyruvate dehydrogenase activity is of crucial importance to the interaction between carbohydrate and lipid oxidation, as this reaction commits pyruvate, derived from glycolysis, to the formation of acetyl groups and eventual complete oxidation to carbon dioxide. There is no pathway for the reformation of carbohydrate from acetyl groups, and so the pyruvate dehydrogenase reaction must be attenuated during the oxidation of fat and inhibited in starvation. In addition, it must respond to the energy-demands upon the tissue, expressed via the ratios of ATP/ADP and $NADH/NAD^+$.

Control of pyruvate dehydrogenase is correspondingly complex but is effected, in part, by covalent modification of the protein. Involved is a cycle of phosphorylation and dephosphorylation, the former process giving rise to a catalytically-inactive protein. The amount of active enzyme (denoted PDH_a) reflects the resultant of the activities of a specific kinase enzyme and a phosphatase, and is sensitive to a change in the activity of either. It was reported previously that Ca^{2+}-ions enhance the activity of the phosphatase thereby leading to a raised content of PDH_a. Preliminary experiments reported last year (Annual Report 1977-78) showed that respiring heart mitochondria respond to changes in the extramitochondrial Ca^{2+}-ion concentration in the range 5.5×10^{-8} M to 4.7×10^{-7} M with large and reversible changes in steady-state PDH_a content. This finding is of interest because these are plausible cytosolic free-Ca^{2+} concentrations and the reversibility of the activation makes it potentially of regulatory significance *in vivo*.

We report now that the dependency of PDH_a content on Ca^{2+}-ion concentration was substantially similar in the presence or absence of palmitoylcarnitine (with pyruvate present at 100 μM in either case), and during resting respiration or respiration stimulated to 50% of the maximal rate. These parameters affect the poise of the interconversion system and could conceivably have affected sensitivity to Ca^{2+}-ions. Moreover, it was substantially similar at 25° and 37°, with the difference that the steady-state was achieved more rapidly at the higher temperature (within 5 min).

Use of a Ca^{2+}-ion-selective electrode showed that respiring heart mitochondria withdraw Ca^{2+}-ions from media down to a concentration of 10^{-7} M. This is in contrast to experiments in another laboratory where liver mitochondria release Ca^{2+}-ions under these conditions. It is noted that the diminished Ca^{2+}-uptake seen at 10^{-7} M (relative to higher concentrations) is not sufficient to give a maximal PDH_a content.

Extensive controls showed that the effect of Ca^{2+}-ions on PDH_a content was not mediated by a decrease in the mitochondrial ATP/ADP and $NADH/NAD^+$ ratios owing to the energy-demand of cation-uptake. The $NADH/NAD^+$ ratio is a particularly sensitive index of mitochondrial energy-status and was shown to be unchanged with Ca^{2+}-ion concentration up to 4.7×10^{-7} M; it was 1.75 during resting respiration with 100 μM pyruvate as substrate. The ratio acetylCoA/CoA, by contrast, increased from 0.64 to 1.6 as Ca^{2+}-ion concentration increased from zero to 4.7×10^{-7} M. This change cannot be a cause of the increased PDH_a content, but rather is likely to be an effect of it, and to maintain constant flux through the pyruvate dehydrogenase reaction in the presence of an increased amount of catalytically-active protein (PDH_a).

It is concluded that the effect of Ca^{2+}-ions is a direct effect at the level of the interconversion enzymes and does not depend upon an alteration of the mitochondrial energy-state. It may well increase flux through this major catabolic pathway in response to an elevated cytosolic Ca^{2+}-ion concentration and increased contractile activity of the heart.

Age-Associated Changes in the Activity of Rate-Determining Enzymes of Oxidative Metabolism - The age-linked changes in the activity of enzymes involved in major catabolic pathways in heart were investigated. This work was stimulated by the finding (reported last year) of a specific decrease with senescence in the activity of several enzymes involved in fatty acid and acylcarnitine oxidation in heart mitochondria and the general paucity of knowledge of the activity of the rate-determining enzymes of oxidative metabolism in tissues from senescent animals. These are the enzymes catalyzing reactions which are far from equilibrium in the cell; *e.g.*, citrate synthase, NAD-isocitrate dehydrogenase and α-ketoglutarate dehydrogenase for the tricarboxylate cycle. Measurements were made in homogenates of rat heart, for the reason that the isolation of a mitochondrial fraction may obscure age-changes, owing to the selective loss of more fragile organelles. The following activities were measured, with the 24-month value being given as a percentage of the 6-month value, on a wet-weight basis: citrate synthase (82%); NAD-isocitrate dehydrogenase (84%); α-ketoglutarate dehydrogenase (81%); cytochrome *c* oxidase (76%); carnitine acetyltransferase (49%) and β-OHacyl-CoA dehydrogenase (73%). Thus, there is a concerted decrease in the activity of tricarboxylate cycle enzymes, which may reflect a lower tissue content of mitochondria, and a greater decrease in two enzymes involved in lipid metabolism. This latter finding is consistent with the findings reported last year on the decrease in the activities of these enzymes in isolated heart mitochondria from the 24-month rat.

Carnitine Transport Systems - Because of the changes in the metabolism of acylcarnitine compounds that occur with age in the heart and the decreased carnitine content of that organ, we initiated a study of the carnitine transport system of the mitochondrial membrane, which shows a decreased activity in heart mitochondria from senescent animals (reported last year). The approach was to solubilize the carrier protein, with a view to the characterization of its physical properties. A first step was the reconstitution of the protein into liposomes of purified phospholipid, allowing the estimation of its transport activity. Use of the cholate-dialysis procedure gave vesicles in which the transport of carnitine was dependent upon the protein extracted from the mitochondria and was inhibited by the sulfhydryl reagent mersalyl, which is very effective in the intact mitochondrion. Solubilization with the non-ionic detergent Triton, followed by protein precipitation with ammonium sulfate solutions, was less successful. However, the solubilization with cholate and reconstitution is less than ideal in that inhibition by mersalyl requires higher concentrations than in the mitochondrion. In addition, counter transport (*i.e.*, stimulation of carnitine entry by the presence of intravesicular carnitine) was not demonstrated, and is a hallmark of this transport system in the mitochondrion. Thus, doubt remains that the transport system has been resolved and reconstituted in its entirety.

Since serum concentrations of carnitine and, in turn, the concentrations in tissues which accumulate the metabolite, are influenced by the reabsorption of carnitine from the glomerular filtrate in the kidney, pilot studies were initiated on the transport of carnitine by renal proximal tubule brush border membrane vesicles. Preliminary findings demonstrating accelerated exchange diffusion of carnitine and our observation that the uptake of carnitine was a function of the intravesicular volume indicate a carrier-mediated transport system in the membrane. Uptake of carnitine was enhanced by the presence of Na^+.

The Role of Ca^{2+} in the Regulation of Mitochondrial Metabolism - In keeping with our long-standing interest in control of mitochondrial oxidation at the dehydrogenase level, in general, and control by Ca^{2+}, in particular, an equation was formulated which enabled the calculation of the concentrations of free-Ca^{2+} as well as those of all ionic and chelated chemical species in a reaction mixture containing calcium, magnesium (or manganese), and free metal chelators, *i.e.*, phosphate, ATP, ADP, citrate, and EGTA. The calculated values of free-Ca^{2+} were compared with those determined experimentally using the Ca^{2+}-specific electrode and the two were found to be in excellent agreement. Such calculations enabled us to examine the significance of the inhibition by Ca^{2+} of the mitochondrial NAD^+-linked isocitrate dehydrogenase. In the absence of effectors, the enzyme was inhibited 50% by 150 μM free-Ca^{2+}, whereas in the presence of ADP, ATP, citrate, and phosphate, the apparent K_I for free-Ca^{2+} was 75 μM.

It was reported, by others, that Ca^{2+} stimulated ketogenesis. This involved liver mitochondria oxidizing fatty acids and the report included a finding that mitochondrial $NADH/NAD^+$ ratio was elevated in response to Ca^{2+}. Such a highly unusual observation suggested to us that the β-oxidation pathway of fatty acid oxidation may have been activated at a dehydrogenase level. In collaboration with Dr. R. Hershcopf we found no evidence for this reductive effect. Only seen was the respiratory stimulation to be expected of an excessive Ca^{2+}-load.

Significance to Biomedical Research and to the Program of the Institute: These studies define the mechanisms whereby age-dependent perturbation in physiological control systems may lead to the inability of the aged organism to maintain homeostasis. This fundamental information is needed for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities.

Proposed Course of the Project: Measure activities and dependence upon age of non-equilibrium enzymes of oxidative metabolism in homogenates of kidneys from rats. Study the interaction of substrate oxidation by heart mitochondria under more physiological conditions of temperature, ATP/ADP ratio, bicarbonate concentration, etc. Characterize the carnitine transport system in renal brush border membrane vesicles.

Publications:

Hansford, R. G. and Cohen, L.: Relative importance of pyruvate dehydrogenase interconversion and feed-back inhibition in the effect of fatty acids on pyruvate oxidation by rat heart mitochondria. Arch. Biochem. Biophys. 191: 65-81, 1978.

Hansford, R. G.: Review on Bioenergetics and Aging. In Handbook on the Biology of Aging, in press.

Bulos, B. A. and Sacktor, B.: Determination of the concentration of free-Ca^{2+} in the presence of magnesium (or manganese) and chelating effectors of the NAD^{+}-linked isocitrate dehydrogenase. Anal. Biochem. 96: 62-72, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00048-05 LMA

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

ATP-Linked Ion Transport Mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	C. Filburn	Research Chemist	LMA NIA
	R. W. Albers	Chief, Neurochemistry Section	LNC NINDCDS
	A. S. Hobbs	Staff Fellow	LNC NINDCDS

COOPERATING UNITS (if any)

Laboratory of Neurochemistry, National Institute of Neurological and Communicative Disorders and Stroke, NIH

LAB/BRANCH

Gerontology Research Center, Laboratory of Molecular Aging

SECTION

Biophysics and Intermediary Metabolism Sections

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.2	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

(1) Studies of the regulation of Ca^{2+} uptake by sarcoplasmic reticulum from young and aged animals were extended to include the effects of treatment with cyclic AMP and protein kinase. Results show that following treatment with cyclic AMP sarcoplasmic reticulum isolated from young hearts exhibits greater activity than membranes prepared from aged hearts. The percent increase in response to cyclic AMP was the same in both age groups indicating that the mechanism for mediating the response is unaltered with age. (2) Measurements of the passive permeability to Ca^{2+} and phosphoenzyme level associated with Ca^{2+} transport in sarcoplasmic reticulum from young and old hearts indicate that differences in maximum velocity of accumulation reflect changes in turnover rate rather than in passive leak or in site density. (3) Comparative investigation of the ATPase partial reactions in the Ca^{2+} and $Na^{+} + K^{+}$ pumps provide evidence that cation-induced slow conformational changes control the overall enzyme activity.

GRC/LMA-158

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: (1) To obtain a clearer understanding of the age-related changes in functional activity of transport systems affecting cardiac performance; (2) to provide a detailed kinetic description of the enzymatic reactions linked to active ion transport.

Methods Employed: Filtration was used to measure Ca^{45} accumulation by sarcoplasmic reticulum (SR) vesicles following treatment with cyclic AMP and protein kinase. The kinetics of ligand-induced transitions in transport ATPases were investigated by means of the rapid mixing acid quench-flow technique.

Major Findings: A decreased velocity of Ca^{2+} transport in isolated sarcoplasmic reticulum (SR) is known to be associated with a prolonged relaxation time in the aging heart. Kinetic analysis of the changes affecting Ca^{2+} transport showed that V_{max} was decreased in the SR from old rat hearts implying that the turnover rate or site density is diminished with age. To test whether the site concentration in SR decreases with age, the level of a transport intermediate which is proportional to the enzyme concentration was measured. The mean level of the intermediate was increased slightly in the young age group as compared to the old but the difference was not significant suggesting that the change in V_{max} is due primarily to a change in the turnover rate rather than in the number of sites.

The contribution of the passive Ca^{2+} leak to net uptake was evaluated by measuring Ca^{2+} efflux from passively loaded vesicles in the presence of an external chelator. Rapid and slow phases of Ca^{2+} efflux were present in young and old SR with no detectable age difference in either component. These results lend support to the concept that an active rather than passive property of the membrane is responsible for the diminished Ca^{2+} transport rates in cardiac SR.

Cyclic AMP and protein kinase treatment of isolated SR vesicles increase their Ca^{2+} transport activity by a mechanism that is postulated to mediate the inotropic response to catecholamines in the intact myocardium. In senescent myocardium the response to catecholamines is diminished compared to that of the young adult heart implying a reduced adaptation to stress with age. In order to investigate the mechanism of this change, the increase in Ca^{2+} transport activity of SR prepared from young and old hearts due to added cyclic AMP and protein kinase was studied with the following results:

(1) At a less than saturating Ca^{2+} level baseline transport activity was higher in the young age group than in the old age group ($p<.05$), as previously demonstrated.

(2) Addition of cyclic AMP and protein kinase to SR increased significantly ($p<.005$) the Ca^{2+} transport activity in both age groups. The percent stimulation above baseline activity (55% in the young as compared to 53% in the old) did not differ significantly with age.

(3) The level of activity after stimulation by cyclic AMP was higher in the

young group than in the old ($p<.05$); however, stimulated Ca^{2+} transport activity in the latter exceeded baseline activity in the young adult group.

These results suggest that the mechanism of the diminished inotropic response in senescent myocardium may be partly explained by a lower velocity of Ca^{2+} accumulation in SR resulting in less Ca^{2+} being sequestered and therefore less released during excitation.

The mechanism for stimulation of Ca^{2+} transport by cyclic AMP in canine cardiac SR involves phosphorylation of a low molecular weight protein constituent which alters the carrier binding affinity for Ca^{2+}. Preliminary results with rat cardiac SR show that it is possible to detect cyclic AMP-dependent phosphorylation and that the levels of phosphorylation are similar in young and old membranes. From these data and the transport studies it is concluded that the major changes affecting the SR Ca^{2+} pump are in the enzymatic carrier and that the system which regulates the activity of the pump in response to hormonal stimuli is basically unaltered with age.

The mechanisms of the transport ATPases include certain ion-dependent conformational transitions which can be monitored indirectly through the effects they have on the chemical enzymatic reactions such as phosphorylation and ATP hydrolysis. The kinetics of transitions occurring in the (Ca^{2+} + Mg^{2+}) and (Na^{+} + K^{+}) activated ATPases were examined in rapid mixing experiments in which the enzyme was premixed with a particular cation and later phosphorylated to follow changes induced by that cation. Addition of a high concentration of Mg^{2+} to SR in the absence of Ca^{2+} followed by rapid simultaneous dilution of Mg^{2+} and addition of Ca^{2+} + ATP gave a slow rate of phosphorylation compared to when Ca^{2+} was added to the enzyme prior to Mg^{2+} + ATP. This suggests that Mg^{2+} stabilizes the enzyme in a conformational state distinct from that which binds Ca^{2+} and which is slowly converted to the latter upon addition of Ca^{2+}. The delay in phosphorylation produced by exposure to high Mg^{2+} is antagonized by high ATP which accelerates the conformational transition between the Mg^{2+} and Ca^{2+}-stabilized states by binding to an allosteric site. Analogous behavior was observed in the (Na^{+} + K^{+})ATPase where Na^{+} acts like Ca^{2+} and K^{+} like Mg^{2+}. Such results lend additional support to the concept that slow conformational transitions involving cation-stabilized states have an important role in regulating enzyme activity under conditions permitting transport of these cations.

Significance to Bio-medical Research and to the Program of the Institute: Studies of the transport systems involved in the maintenance and regulation of excitation-contraction coupling in cardiac muscle will provide a clearer understanding of the biochemical changes responsible for alterations in baseline mechanical performance and response to stress that occur with advanced age.

Proposed Course of the Project: Examination of the enzymatic pathways linked to ion transport and changes affecting transport with age will be continued using fast kinetic methods. Collaborative studies with the Cardiovascular Section (CPB) of the effects of interventions on SR function will be initiated to determine the extent of correlation between prolonged contraction duration in muscle and the diminished Ca^{2+} transport activity of isolated SR.

Publications:

Srivastava, R. C., Froehlich, J. and Eichhorn, G. L.: The effect of platinum binding on the structure of DNA and its function in RNA synthesis. Biochimie 60: 879-891, 1978.

Sumida, M., Wang, T., Mandel, F., Froehlich, J. P. and Schwartz, A.: Transient kinetics of Ca^{2+} transport of sarcoplasmic reticulum: a comparison of cardiac and skeletal muscle. J. Biol. Chem. 253: 8772-8777, 1978.

Froehlich, J. P.: The effect of pretreatment with calcium and magnesium ions on phosphoenzyme formation by sarcoplasmic reticulum ATPase. Biophys. J. 24: 61-62, 1978.

Gerstenblith, G., Spurgeon, H. A., Froehlich, J. P., Weisfeldt, M. L. and Lakatta, E. G.: Diminished inotropic responsiveness to ouabain in aged rat myocardium. Circ. Res. 44: 517-523, 1979.

Froehlich, J. P., Albers, R. W. and Hobbs, A. S.: Kinetics of ligand-induced transitions between the E_1 and E_2 conformations of (Na^+, K^+)ATPase studied by the quench-flow technique. In Proc. 2nd Int. Conf. on the Properties and Functions of (Na^+, K^+)ATPase, in press.

Guanieri, T., Spurgeon, H. A., Froehlich, J. P. Weisfeldt, M. and Lakatta, E. G.: Diminished inotropic response but unaltered toxicity to acetylstrophanthidin in the senescent beagle. Circ. Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00049-01 LMA

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies on the Oral Physiological Status of Man During Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	W. H. Bowen	Dentist	NCP NIDR
	M. F. Cole	Visiting Scientist	NCP NIDR

COOPERATING UNITS (if any)

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LAB/BRANCH

Gerontology Research Center, Laboratory of Molecular Aging

SECTION

Intermediary Metabolism Section

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.65	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

There has been little systematic investigation of tissues within the aging oral cavity. It is the purpose of this project to assess the oral physiologic status of participants in the Baltimore Longitudinal Study and thus provide baseline information which is, at present, essentially lacking. The study has two major objectives: (1) to define subjects acccording to their dental and oral health; to examine biological factors which are likely to influence oral health. The latter include studies of salivary gland function (saliva output: quantity and constituents) and studies on dental plaque (bacterial population and plaque fluid components).

Project Description:

Objectives: This project is designed to describe oral and dental tissues during human aging as well as to evaluate various biological factors which affect oral health. In order to begin to understand the status of the oral cavity with increased age, it is important to have both detailed clinical and biochemical/physiological information from subjects. One can then correlate clinical dental situations with specific processes or events. Furthermore, because of the nature of the Baltimore Longitudinal Study, we can examine, with time, the influence of medical status, medication, psychological and sociological factors, etc., on the oral health of the participants.

Methods Employed: All subjects studied (currently about 1/3 of presenting participants) undergo a detailed oral and dental examination. This includes the following procedures: (1) comprehensive history: covered are past treatment, oral hygiene habits, pertinent nutritional information, present complaints, etc.; (2) physical examination of oral soft tissues (mucosa, salivary glands, tongue, palate, floor of mouth, etc.); (3) examination of oral motor functions (positional stability, swallowing, mastication) by study of the facial, tongue, palatal, supra and infrahyoid musculature; (4) clinical assessment of dental caries experience; number of decayed, missing and filled tooth surfaces and teeth; (5) determination of periodontal health status using the Ramfjord periodontal disease index for examining teeth 3, 9, 12, 19, 25, and 28; and (6) radiographic examination by using two dental bite-wing and one panographic radiographs. The former type is particularly useful for determining non-clinically detectable caries while the latter is helpful in detecting osseous pathology. Copies of all radiographs, along with a summary of any clinical findings, are sent to the participants private dentist.

The biological factors assessed in each individual are: (1) the nature and amount of dental plaque present; and (2) salivary gland function and saliva composition. Dental plaque is studied in two ways: a) two samples of plaque are collected in discrete locations of the same tooth (one a cervical, the other a coronal locale). The kind of bacteria present in each sample is then evaluated by conventional microbiological techniques; b) the remaining plaque is then collected by gentle scraping with a dental instrument and "pooled." The wet weight of this sample provides an index of oral hygiene. Plaque is then centrifuged and a resultant sediment and fluid phase obtained. The supernatant (plaque fluid) is then examined by immunological and biochemical techniques to assess the content of various specific proteins (including immunoglobulins A, G and M, lysozyme, lactoferrin, and lactoperoxidase).

Particular emphasis is placed on saliva composition, since it is now well accepted that saliva functions to protect the teeth and oral mucosa. Some specific roles for saliva in oral health include mechanical cleansing of teeth; buffering action neutralizing acids formed by dental plaque; maintenance of taste acuity and sensation; lubrication of the oral mucosa; providing the oral cavity with several protective anti-bacterial proteins; and maintaining tooth integrity (minimizing enamel surface dissolution).

Unstimulated whole saliva and stimulated parotid saliva (2% citric acid is used to stimulate) are collected from each individual. All samples are examined for flow rate; total protein; "protein profile" determined by gel electrophoresis; and by immunologic and biochemical techniques to assess the content of several anti-bacterial proteins (same as with plaque fluid above). Also, in parotid saliva the concentration of several electrolytes is determined (sodium, potassium, calcium, phosphorous). This provides information on transport processes occurring in duct membranes. Other examinations include amylase content and isozyme profile and the concentrations of other specific salivary proteins including the proline rich proteins and statherin.

Major Findings: Since this project is just completing its first year, results should be interpreted cautiously. At this stage data handling and analysis methods are still being worked on, thus making early approaches relatively primative. Also, many of the biological factors studied for first year samples are not yet fully analyzed. Despite these qualifications there have been many observations worthy of note.

Particularly interesting is the simple observation that most participants in the Baltimore study have functioning natural dentitions. Few of our subjects (<10%) are edentulous. Generally, it is considered that most older individuals have lost their teeth and thus prosthetic problems are the principal dental concern of the aging and aged. Clearly this is not a general case. Further, with improvements in dental care and the current focus on preventive techniques, one can expect that, in the future, most older individuals will retain a functioning natural dentition.

However, with the retention of their natural teeth, older individuals must now be concerned with both dental and periodontal disease. We have observed in this population, a marked prevalence of cervical dental caries (root caries) in older individuals. This type of decay is present (both active and restored caries considered) in 29.3% of our subjects under age 50 while in individuals greater than age 50 the prevalence is 87.9%. The factors causing cervical caries have not been elucidated. With our study design we should be able to help clarify the etiology since bacterial populations, salivary anti-bacterial protein levels, and salivary electrolyte composition are being examined.

The study of the oral motor apparatus has begun to yield information about important daily life functions and their status with increasing age. The study has thus far focused on postural functions of the oral musculature as well as swallowing and chewing. A high prevalence of some decrement in each of these functions, to varying degrees, has been observed with increased age. Particularly noticeable has been some alteration in labial posture which leads to a problem of labial spill of saliva or drooling. A deficiency in control of labial posture is fairly common in older subjects, less so in middle-aged individuals and infrequently observed in younger participants. In addition to the annoyance or embarassment, drooling may frequently result in a chronic inflammation of the labial fornices.

Positional stability of the tongue may also decrease in middle-aged and older individuals. Some impairment of this function was observed with moderate frequency in the older and mid-age groups but infrequently in the younger subjects. Dysfunction of the tongue suspension muscles could manifest itself as: a) a type of sleep apnea where the tongue, due to lack of support, could occlude the pharyngeal airway while an individual slept on his back; or b) increased mouth breathing due to an incompetent closure of the junction of mouth and pharynx.

Swallowing and chewing functions were also noted to be somewhat impaired in middle-aged and older individuals. Particularly important would be perturbations in the swallowing mechanism which might lead to potentially disasterous consequences (fatal choking).

Initial results of studies on saliva and salivary gland function have been quite interesting. Generally, it is considered that saliva production decreases with age, particularly so among post-menopausal females. In our studies of parotid saliva flow, attempts have been made to control for a number of variables including diurnal variation, functional variation and stimulus utilized. Among all females we observe a tendency for lower flow rates (average 25% decrease) in subjects greater than 50 years old. However, when subjects with questionable medical status (on medication at present, endocrinopathies, etc.) are excluded from the analysis, differences are not apparent. These results are certainly preliminary and more "healthy" older females need to be examined before any conclusion regarding the relationship of age or pathology to decreased salivary flow can be reached. Among male participants no tendency towards decreased salivary flow in older individuals ("healthy" and of questionable medical status) has been observed.

Studies of salivary gland function, *i.e.*, output of various constituents and their relation to flow rate, has yielded particularly interesting information. The analyses thus far have focused on individual differences among "healthy" participants. In examining Ca^{2+} and Na^{+} output (both of which are positively correlated with flow rate) and correcting data for flow, a fairly narrow range of values is seen up to about age 55. Thereafter, the "scatter" of individual values is much more frequent suggesting some increase in "altered parotid duct function" in these people. Generally, our findings were similar for both men and women.

Data on other electrolytes, such as potassium (concentration is independent of flow) and phosphorous (concentration is negatively correlated with flow) are not yet analyzed but should provide other "windows" on salivary gland function. Additionally, by looking at discrete protein concentrations in saliva, another dimension of gland physiology can be studied. Our analyzes include secretory products of both acinar cells (such as amylase, proline rich proteins) and ductal cells (lysozyme).

Significance to Bio-medical Research and to the Program of the Institute: This study has begun to define the physiologic status of the oral cavity in men and women of various ages. The loss of the dental apparatus (teeth, gingiva, periodontium) can have marked adverse effects on facial appearance, speech and dietary patterns. Also, these dental alterations could have a negative effect on the self-image or sense of well-being of the aging individual. Precise orofacial motor function, as noted above, is necessary for basic daily life functions. Changes in oral motor function could certainly affect the quality of one's life as well as potentially being a threat to life itself. By providing basic descriptions of oral physiological functions with age, this study will allow rational assessments of what impact changes in specific functions have on oral health. Such an understanding is a necessary pre-requisite for devising procedures to limit or delay age-related changes in oral tissues.

Proposed Course of the Project: Examinations of oral tissues as described above will continue. Studies of individual salivary gland function will be expanded to include the study of submandibular/sublingual gland products and will consider both stimulated and unstimulated parotid gland saliva. Measurement of taste acuity, using threshold and magnitude scaling procedures, will be introduced to investigate more precisely change in this important oral sense. On a sub-set of subjects quantitative studies of tongue function and support, using ultrasound techniques, will be initiated. Studies on dental plaque will continue in the present format.

Publications:

Baum, B. J.: Salivary glands and saliva: recent advances in understanding their function and role in oral health. The New Dentist 9: 21-26, 1979.

Baum, B. J.: Aging and oral motor function. In Oral Motor Behaviors: Impact on Oral Conditions and Dental Treatment, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00050-01 LMA

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Regulation of Salivary Gland Function During Increased Age

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. J. Baum	Dental Officer	LMA NIA
OTHERS:	C. Filburn	Research Chemist	LMA NIA

COOPERATING UNITS (if any)
None

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Gerontology Research Center, Laboratory of Molecular Aging

SECTION
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INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.45	OTHER: 0.35

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous clinical observations have suggested that with increased age, decremental changes in salivary gland function occur. It is the purpose of this project to investigate, in an animal model, biochemical factors which regulate salivary gland function and study their operation and regulation with increased age. Initial focus has been on mechanisms involved in protein secretion from the rat parotid gland, particularly the role of β-adrenergic receptors, cyclic AMP and cyclic AMP-dependent protein kinase.

Project Description:

Objectives: These studies have as their major objective to understand the biochemical mechanism of protein secretion in salivary glands (*i.e.*, release of proteins into saliva) and alterations in this mechanism during the aging process. Many salivary proteins directly protect oral soft and hard tissues, thus oral health can be related to production of saliva.

Methods Employed: The rat parotid gland *in vitro* is utilized as a model tissue. β-Adrenergic receptor stimulated protein secretion is studied using β-adrenergic agonists and antagonists. Protein secretion is monitored by release of amylase and/or previously radiolabelled protein. Cyclic AMP stimulated events, subsequent to β-adrenergic agonist binding, are followed by measuring activation ratios of cyclic AMP dependent protein kinase. Cyclic AMP dependent and independent protein kinases, from membrane bound and cytosolic fractions of parotid acinar cells, are characterized by anion exchange chromatography and kinetic parameters.

Major Findings: Adult (6 mos) and aged (24 mos) male rats respond to simple β-adrenergic stimuli of protein secretion. At saturating concentrations of isoproterenol the rates and maximal levels of secretion (30 min) are similar for the two age groups. In both age groups, stimulation of β-adrenergic receptors leads to a brisk elevation in the activity of cyclic AMP dependent protein kinase. This activity reaches a maximum in 5 min then gradually begins to diminish. Cyclic AMP dependent protein kinases from parotid gland have been partially characterized. Both membrane and cytosolic fractions contain principally the type II form of the enzyme (>90%) with the remaining activity appearing to be a type I form. Greater than 80% of the total enzyme activity can be recovered from the cytosolic fractions.

Significance to Bio-medical Research and to the Program of the Institute: These studies begin an examination of the mechanisms regulating secretion from the parotid gland in adult and aged animals. The exocrine product of salivary glands (saliva) contains many specific proteins whose primary function is the maintenance of oral health. Salivary constituents thus represent a major class of host factors likely to influence dental disease experience. In aged individuals, decrements in salivary gland function have been observed. These changes would likely have a negative influence on the health of oral tissues. By understanding the biochemical mechanisms controlling salivary gland secretion, one can begin to recognize any "physiological malfunction" present and conceivably then design therapeutic means to alleviate the disorder.

Proposed Course of the Project: Further studies will focus on the immediate consequences of β-adrenergic stimulation. This includes the identification of physiological substrates for cyclic AMP dependent protein kinase; determination of what, if any, role these substrates have in mobilizing protein secretion; and examination of the role of ionic calcium and calmodulin (calcium dependent regulatory protein, CDR) in protein secretion. In addition, the regulation of new "secretory" protein synthesis, following β-adrenergic agonist induced secretion, will be investigated.

Publications:

None

Laboratory of Neurosciences

The Laboratory of Neurosciences at the National Institute on Aging was formed in 1978, and has embarked on a program of research on the central and peripheral nervous systems and muscle, in health, disease and aging. This report discusses the following projects: (A) Blood-brain barrier and central nervous system function, (B) Cerebral metabolism, relation to brain function and aging, (C) Function of peripheral nerve and muscle, (D) Pharmacology of central and peripheral catecholaminergic nervous systems, (E) Synapse development, specificity and mechanism in culture and (F) Central nervous system binding sites for the neurotoxin kainic acid. I am primarily responsible for Project A, Dr. E.D. London for Projects B and F, Dr. N.L. Shinowara for Project C, Dr. C.C. Chiueh for Project D and Dr. J.M. Thompson for Project E.

A. Blood-Brain Barrier and Central Nervous System Function.

Studies of blood-brain interaction in health, disease and aging require quantitative measurements of cerebrovascular permeability and transport, and adequate pharmacokinetic theories to interpret drug distribution within the various brain compartments in relation to plasma concentrations and metabolism. Current techniques for measuring cerebrovascular permeability and transport are insensitive, semi-quantitative and can be influenced by changes in cerebral blood flow. We therefore elaborated and tested, experimentally and theoretically, a new quantitative procedure that can be used in conscious animals, and that is independent of cerebral blood flow and at least 1000 times more sensitive than any other currently available technique.

We used this procedure to measure ^{14}C-sucrose permeability at cerebral blood vessels of mature and senescent Fisher 344 rats. Sucrose enters the brain very slowly, and a slight alteration of cerebrovascular integrity would easily be noticed as an increase in permeability. We found, however, that ^{14}C-sucrose permeability was not changed in senescent rats. Thus, we could not support the hypothesis (at least for the rat) that aging of the brain is caused by entry of blood-borne, brain-reactive antibodies across a defective blood-brain barrier.

A major problem in psychopharmacology is that a drug response often cannot be related directly to drug dosage or plasma concentration. In the elderly, furthermore, the high incidence of neurotoxicity may be caused by a high brain concentration of a drug at a dosage that is not toxic in young individuals, or by altered receptor sensitivity to a drug. Using our method as discussed above, we established a general relation between cerebrovascular permeability and the octanol/water partition coefficient of a drug. We also formulated a multi-compartment (brain-plasma) kinetic model that incorporates the observed relation between permeability and partition coefficient, and that can be used to predict brain concentration of a psychoactive drug from the dosage and plasma concentration.

We used our pharmacokinetic procedure and analysis to demonstrate that synthetic opioid peptides are significantly permeant at the blood-brain barrier.

Prior to our studies, other methods suggested that they were not. Our findings suggest that feedback may operate between circulating peptides that have potential central nervous system effects and brain sites that regulate their release into the systemic circulation.

In 1972, we first demonstrated that the blood-brain barrier could be reversibly opened in animals by infusing a hypertonic solution of a water-soluble nonelectrolyte (e.g. urea, mannitol, arabinose) into the internal carotid artery. The effect later was shown to be caused by osmotic shrinkage of cerebrovascular endothelial cells, with consequent widening of interendothelial tight junctions. In the last years, we experimentally refined the osmotic method in the rat, and quantified changes of cerebrovascular permeability in relation to infusate concentration and infusion time, as well as in the recovery period. We showed that, when the barrier opens transiently, brain metabolism is stimulated and a temporary brain edema is produced, and thus that barrier integrity must be maintained continuously for normal brain function.

We also employed the osmotic method as a pharmacological tool. We demonstrated in rats and dogs that osmotic barrier opening can be used to increase brain uptake of methotrexate, an anticancer agent, to a sufficient extent so as to treat, in principle, certain sensitive brain tumors. We also used the osmotic method to allow exogenous enzymes (e.g., α-mannosidase) into the rat brain, and showed that they are taken up within neuronal lysosomes where they retain their activities for days. These studies eventually may be applied to man, to treat metastatic brain tumors in the former case, and for enzyme replacement therapy in lysosomal storage diseases in the latter.

On the basis of our observations that osmotic barrier opening produces a transient brain edema, we formulated a comprehensive model of vasogenic brain edema that can be used to analyze the quantitative contribution of such factors as tissue and plasma osmotic and hydrostatic pressures, tissue compliance and capillary and tissue hydraulic conductivities. The model predicts the intensity and time course of brain edema from these parameters, and indicates that vasogenic edema might be treated by reducing capillary permeability and hydraulic conductivity, reducing brain compliance or, in some cases, by reducing cerebrospinal fluid pressure. As edema is a major complicating cause of death in stroke and brain trauma in man, these therapeutic principles may eventually help to reduce mortality due to edema.

B. Cerebral Metabolism, Relation to Brain Function and Aging.

Regional cerebral glucose consumption and regional blood flow reflect the functional state of the brain. With aging in man, blood flow appears to decrease in the frontal lobe and increase in the parietal lobe in the absence of evidence of cerebrovascular disease, and mean brain metabolic rate may be unchanged. Dr. London employed the ^{14}C-2-deoxy-D-glucose method of Sokoloff *et al*. (1977) to examine regional rather than overall rates of cerebral glucose consumption in Fisher 344 rats, at 3, 12 and 24 months of age. She wanted to evaluate age-related alterations in functional states in specific brain regions, because of published studies on age related changes in neurotransmitter systems. Regional glucose consumption was found to decline with age in some but not all brain regions in Fisher 344 rats. Structures which showed no significant decrements were cerebellar gray and white matter, nucleus accumbens, septum, hypothalamus and thalamus. Structures which showed reductions of the order of 20-30% were the superior colliculus, inferior colliculus, medulla, midbrain, caudate nucleus and frontal and lateral cortex. Most changes occurred, however, between 3 and 12 months rather than between 12 and 24 months.

We also showed that regional cerebral glucose consumption was markedly increased in most areas of the cerebral cortex during spreading cortical depression, a convulsion-like state which is induced in the rat by applying potassium to the cortical surface. Many subcortical structures had a reduced consumption. Cortical glucose utilization remained elevated during recovery, but increased metabolic activity was distributed in perpendicular cortical columns.

We also developed techniques to measure regional cerebral blood flow in conscious animals, and evaluated ^{14}C-antipyrine, ^{14}C-iodoantipyrine and ^{3}H-nicotine as experimental tracers. Blood flow usually is coupled to cerebral metabolism, and as such is an indirect measure of the functional state of the brain or, if uncoupled, of the severity of brain pathology. We showed that ^{14}C-iodoantipyrine is a better indicator of flow than is ^{14}C-antipyrine, and also demonstrated for the first time that ^{3}H-nicotine can be employed to measure flow.

C. Function of Peripheral Nerve and Muscle.

In order to understand regulation of peripheral nerve function by the blood-nerve barrier, we perfused an excised cylindrical portion of the frog sciatic nerve perineurium (a sheath that surrounds nerve axons), and measured permeability and electrical properties of the perineurial wall. Low permeabilities of ^{14}C-sucrose and of Na^+, Cl^- and K^+ demonstrated that the perineurium represents a major restriction to blood-nerve exchange. There is a selectivity to K as compared to Na^+. Furthermore, the frog perineurium does not appear to actively transport either Na^+ or K^+. Perineurial permeability can be increased reversibly by 10% stretch or irreversibly by further stretch or hypertonicity. We also examined the morphological basis of the barrier properties of the perineurium. Dr. Shinowara demonstrated that the frog perineurium has 7 to 19 layers of flat cells which are connected by desmosomes and tight junctions. Electron dense tracers (horseradish peroxidase, lanthanum) are prevented from penetrating the perineurium by an inner layer of cells that are connected by tight junctions. Perineurial cells have numerous vesicles, most of which are fused to the external and internal cell membranes to form pits. In some rare cases, channels extend across an entire cell.

D. Pharmacology of Central and Peripheral Catecholaminergic Nervous Systems.

Dr. C.C. Chiueh's work is concerned with the effect of aging, stress, and antihypertensive drugs on the neuronal activities of both the central and peripheral catecholaminergic nervous systems, and on the function of the cardiovascular system in the rat. An age-dependent increase in blood pressure was found in Fisher-344 rats, Wistar rats and spontaneously hypertensive rats. Immobilization stress induced increases in plasma catecholamines, corticosteroid and blood glucose, but the increases were not related to age. However, the aged rats appeared to release these substances into the circulation more slowly than did the younger groups. In physiological measurements, immobilization stress produced an α-adrenergic vasopressor response in young rats but a β-adrenergic vasodepressor response in old rats, which suggests that there is an age-related change in α-adrenergic receptors in rats. Furthermore, older rats usually died following stress. Dr. Chiueh also found that the age-dependent, increase in blood pressure could be blocked by administration of Uncarine A, the active ingredient of a Chinese herbal medicine. Uncarine A also reduced the blood pressure of spontaneously hypertensive but not of normotensive animals, for up to 48 hr. His findings suggest that it may eventually be of use in treating hypertension in man.

E. Synapse Development, Specificity and Mechanism in Culture.

Dr. J. Thompson demonstrated that synapse formation, synapse termination, synapse specificity and neurotransmission can be studied using neurons and muscle cells in tissue culture. He detected functional synapses by inserting microelectrodes into muscle cells, and recorded miniature end plate potentials in these cells. He demonstrated that neurons from retina or chick spinal cord form long-lived synapses with muscle cells in culture. Each type of neuron, therefore, possesses a synapse-competent state during its development, when it can form abundant synapses with muscle cells in culture. Dr. Thompson's results suggest a mechanism of synapse specificity that is based on stabilization of correct synapses which is coupled with a synaptogenic period during development for each type of neuron. Dr. Thompson's studies also demonstrate that synapse formation and retention can be studied in a controlled fashion in tissue culture. His findings may be critical for our interpretation of neuronal cell death in the aging nervous system.

F. Central Nervous System Binding Sites for the Neurotoxin Kainic Acid.

In addition to examining regional cerebral metabolism in relation to aging (see above), Dr. E. London examined central nervous sytem binding sites for the neurotoxin, kainic acid, which appears to be specific for neurons that contain glutamic acid receptors. She found maximal binding in brains of the frog, dogfish, goldfish and chick, to both high and low-affinity receptors. In mammals, maximal binding occurred in the forebrain, moderate binding in the cerebellum, and little binding in the medulla. The widespread distribution of highly specific binding sites in neural tissue of different species, together with the observation that binding characteristics are similar, suggest that there is an endogenous neuronal system that has not changed appreciably through evolution.

Dr. London also noted cooperative effects between L-glutamate and kainate at the receptor level. When taken with the observation that kainate is not toxic when glutamatergic innervation is removed, the results suggest that, at the receptor level, kainate sensitizes neurons to the neurotoxicity potential of glutamate-induced depolarization. The demonstration of binding sites in human brain, with kinetic characteristics similar to those in the rat model of Huntington's disease, indicates that kainate's neurotoxicity may be relevant to human disease. The caudate nucleus and putamen, which are severely affected in Huntington's disease, exhibit the highest densities of high affinity kainate-binding sites in the human brain. Dr. London's findings have led her to examine kainate binding in control and Huntingtonian postmortem brains, to see if a change in binding is related to the disease process.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00120-02 LNS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Blood-Barrier and Central Nervous System Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	M. Ohata	Visiting Fellow	LNS	NIA
Others:	K. Ohno	Neurosurgeon	LNP	NIMH*
	W.K. Fredericks	Biologist	LNS	NIA
	L. Sokoloff	Chief	LCM	NIMH
	J.A. Barranger	Neurologist	DMN	NINCDS
	R.O. Brady	Chief	DMN	NINCDS
	K.D. Pettigrew	Statistician	B	NIMH
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NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 2.6	PROFESSIONAL: 1.7	OTHER: .9

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A new method was developed to measure cerebrovascular permeability to poorly permeable as well as rapidly permeable substances. Pharmacokinetic principles for the central nervous system were established that demonstrate in rats that cerebrovascular permeability of nonelectrolytes and organic electrolytes is related linearly to the octanol/water partition coefficient. Synthetic opioid peptides are significantly permeable at the blood-brain barrier. In senescent rats, studies with ^{14}C-sucrose show that cerebrovascular integrity is maintained, contrary to the immune hypothesis for brain aging. Barrier permeability can be increased by 10 to 20 fold, by infusing a hypertonic solution of arabinose or mannitol into the carotid artery of rats or dogs. The osmotic method transiently elevates brain water and regional brain glucose consumption, but does not produce long-term cerebral pathology. As a pharmacological tool, the osmotic method has been used to increase brain uptake of methotrexate (an anti-tumor agent) and exogenous enzymes (for use in enzyme replacement therapy). A theory of brain edema was presented to interpret brain water changes following increased barrier permeability, and provides a rational approach to treatment of edema.

PHS-6040
(Rev. 10-76)

Objectives: The blood-brain barrier at the cerebral vasculature regulates the ionic environment of the nervous system, has specific mechanisms for transport of amino acids and glucose that support cerebral metabolism and neurotransmitter and protein synthesis, and prevent access to the brain of water-soluble drugs and other agents. The aims of this project are as follows: (a) to describe the ultrastructure of the blood-brain barrier in relation to its permeability and physiologic functions in normal and aging brain, (b) to determine the quantitative rules that govern drug entry into the brain from plasma, (c) to develop a method to reversibly modify barrier permeability, and to apply it to understanding the central actions of systemically-administered drugs and other agents, and (d) to determine how the barrier regulates behavior and brain metabolism.

Methods Employed: A number of different methods were employed in these studies. Surgical procedures were used to open the skull and dura of anesthetized animals, and to catheterize blood vessels for infusion and plasma sampling. Neurological examinations and behavioral tests evaluated overall brain function and localized deficits. Histological methods were used to examine brain pathology, and electronmicroscopy was employed to evaluate transfer mechanisms at the barrier and barrier ultrastructure. Radioisotope techniques including autoradiography were used to quantitate drug and metabolite transfer from blood to brain. Computers were used to analyze data and to generate relevant models.

Major Findings: A. Quantitative Aspects of Drug Entry into Central Nervous System.

1. Central nervous system pharmacokinetics. The blood-brain barrier limits exchange of drugs between plasma and brain, and makes it difficult to interpret dose-response relations of centrally-acting drugs. We therefore decided to establish empirical rules to quantitatively predict drug entry into the brain from the plasma concentration and the physical propeties of the drug (e.g., lipid solubility). Radiotracers of organic electrolytes and of nonelectrolytes, with differing octanol/water partition coefficients, were injected intravenously in conscious rats. Arterial plasma concentrations were followed, and regional brain concentrations of tracer were determined at various times after injection. A model for blood-brain exchange was formulated and applied to the data. Calculated cerebrovascular permeability was found to be related linearly to the octanol/water partition coefficient of a solute. For a drug, whose partition coefficient is known, the rate of brain accumulation can be predicted now from the linear relation and the history of the plasma concentration. Cerebral blood flow, which was measured in a separate series of experiments with ^{14}C-iodoantipyrine, must also be known for predicting brain accumulation of very permeable drugs (Ref. 1).

2. Entry of opioid peptides into central nervous system. Our new method for measuring permeability and predicting drug entry into the brain is at least 1000 times more sensitive than any currently available (Indicator Dilution Technique or Brain Uptake Index technique) and should be applicable to

pharmacokinetic studies in man. In conscious rats, it was used to show that synthetic opioid peptides -- [D-Ala^2]-methionine enkephalin amide, [D-Ala^{62}, ^{14}C-Homoarg]-β-endorphin, [D-Ala^{62}, ^{14}C-Homoarg^{69}]-β-lipotropin 61-69 and [D-Ala^2, ^{14}C-Homoarg^9]-α-endorphin - which have been reported by other less sensitive methods not to be permeable at the blood-brain barrier, have significant cerebrovascular permeability coefficients which should allow these peptides to fill the brain extracellular space with half-times of 4-12 min, following a step increase in plasma concentration. Our findings suggest that central effects to systemically administered peptides can be explained on the basis of their entry into the brain at the blood-brain barrier, and suggest, furthermore, that feedback may operate between circulating peptides that have potential central effects and brain sites that regulate their release into the systemic circulation. An abstract of this work is in press (Rapoport, S.I., W.A. Klee, K.D. Pettigrew and K. Ohno. Entry of opioid peptides into the central nervous system. Abstr. Ninth Annual Meeting Neurosciences Soc., 1979).

B. Reversible Modification of Blood-Brain Barrier Permeability.

1. Methods and mechanism. The blood-brain barrier at cerebral capillaries is due to a continuous layer of endothelial cells that are connected by tight junctions. We demonstrated that the barrier can be opened reversibly by infusing a hypertonic solution of a water-soluble solute (e.g., arabinose) into the internal carotid artery of rats, rabbits or monkeys. We also showed, with electronmicroscopy and by theoretical models, that barrier opening probably is caused by shrinkage of cerebrovascular endothelial cells and widening of interendothelial tight junctions. The method should prove useful as a tool for studying central actions of drugs that normally do not penetrate the blood-brain barrier (Ref. 2 and 3).

Osmotic barrier opening can be accomplished in animals without producing long-term changes in behavior, or in brain water and electrolytes. However, if the procedure is employed in conscious rats, then transient changes occur that are reversed within 2 to 24 hr. Brain water is elevated by about 1%, due to barrier opening to salts and loss of the protective barrier action against brain edema, and cerebral blood flow no longer is tightly coupled to cerebral metabolism. Finally, behavioral and cerebral metabolic changes reminiscent of convulsions (some changes are prevented by Valium) are produced by barrier opening. Regional uptake of ^{14}C-2-deoxy-D-glucose is increased. This tracer is a glucose analogue that is transported and phosphorylated like glucose within the brain but not metabolized further (Ref. 4-6).

2. Quantification and time course. In order to apply the osmotic method to studies of CNS pharmacology in animals, and possibly to regulating drug entry into the brain in man, it was important to determine thresholds for infusate concentration and infusion time, that produce maximum opening, as well as the time course of such opening. To do this, we employed the quantitative methodology that was discussed above, and used ^{14}C-sucrose as an intravascular tracer to measure and quantify osmotically-induced cerebrovascular permeability changes in the rat.

Retrograde external carotid infusion of a hypertonic arabinose solution reversibly increased cerebrovascular permeability to ^{14}C-sucrose. PA (product of permeability and capillary surface area) rose from 11 x 10^{-6} sec^{-1} in control brains to above 200 x 10^{-6} sec^{-1} in experimental brains. The rise correlated with an increased brain staining by intravascular Evans blue-albumin, and was accompanied by a transient 1-1.5% increase in brain water content. At least 20 sec of carotid infusion was required for 1.6 m arabinose solution to effectively increase cerebrovascular permeability. Prolongation of infusion time or elevation of infusate concentration had no further effect. The increased permeability remained for about 1 hr, during which time it is suggested that drugs of pharmacological interest should be administered for maximum central effectiveness (Ref. 7).

3. Pharmacological uses of reversible osmotic barrier opening. Osmotic barrier opening has been employed by us to allow the following normally-excluded agents into the brain:

a. Enzymes α-mannosidase and horseradish peroxidase, in animal studies of enzyme replacement therapy. The blood-brain barrier of rats was opened reversibly by infusing a hyperosmotic solution of arabinose into the external carotid artery. Permeability was increased maximally in the first 15 min and remained slightly elevated at 1 hr. Osmotic barrier opening significantly increased brain uptake of intravenously injected α-mannosidase (α-D-mannoside mannohydrolase, EC 3.2.1.2.4)(derived from human placenta) and horseradish peroxidase (donor: hydrogen-peroxide oxidoreductase, EC 1.11.1.7). By injection of 4 X 10^{5} units of α-mannosidase into an animal, brain activity rose to about twice the normal control activity of the enzyme. After 30 min, activity of administered enzyme in the extracellular space of the brain was calculated to be 30% of the serum concentration. Biochemical and histological studies with horseradish peroxidase showed that exogenously administered enzyme entered the brain extracellular space immediately after barrier opening and was incorporated within neuronal lysosomal packets during the next 24 hr. Measurable peroxidase activity was found in brain as much as 72 hr after osmotic treatment. The results demonstrate that the blood-brain barrier can be reversibly opened to enzymes, that a glycoprotein enzyme is incorporated into neuronal lysosomes, and that the brain may now be considered a potential target for enzyme replacement therapy in heritable metabolic disorders (Ref. 2).

b. Methotrexate, for therapy of brain tumors. One of the major problems in cancer chemotherapy is the inability of systemic drugs with proven effectiveness to affect metastatic brain tumors, because of the blood-brain barrier. It would be extremely important, especially in cases of single brain metastases in association with pulmonary or breast carcinoma, to be able to open the blood-brain barrier so as to allow proven effective chemotherapeutic agents into the brain.

We performed animal experiments to quantify methodology and effectiveness of osmotic barrier opening as a possible adjunct to brain chemotherapy in man. In anesthetized rats, the blood-brain barrier to ^{3}H-methotrexate was opened by infusing 1.6 m arabinose solution into the external carotid artery, in a retrograde direction, for 30 sec. PA (product of cerebrovascular permeability and capillary surface area), as measured by methods that we developed (see above), increased from a mean of 3.3 x 10^{-5} sec^{-1} in brains of control rats to as much as 29 x 10^{-5} sec^{-1} at the cerebral hemisphere ipsilateral to carotid infusion. When tracer was introduced into the carotid artery after osmotic treatment, brain uptake was increased by a factor of 50 (a factor of 7 due to elevation of PA, multiplied by 7 due to infusion by the carotid route), as compared to uptake by normal untreated brain with infusion into a peripheral vein. The study shows that osmotic barrier opening followed by carotid drug administration should be of use in chemotherapy of the central nervous system.

Carotid infusion of hypertonic mannitol solution was used to open the blood-brain barrier to methotrexate in dogs. Ipsilateral methotrexate brain levels ranged from 90-216 ng/g tissue following intraarterial injection of drug and saline infusion into the carotid circulation, whereas a mean value of 1420 ng/g was measured with carotid infusion of mannitol. Brain levels remained high for 6 hrs. There were no neuropathological changes 24 or 72 hrs after infusion, or up to 2 weeks later. The methotrexate levels reached under these conditions would be sufficient to treat certain cerebral tumors. An abstract of this paper has been published (E.A. Neuwelt, S. Hill, S. Rapoport, S. Sheehan, M. Mayhood, E.P. Frenkel, J. Kirkpatrick, W.K. Clark, Abtracts American Association for Cancer Research (in press).) In dogs, furthermore, blood-brain barrier opening was monitored in vivo by computerized tomography, following intracarotid mannitol. The quantification of in vivo opening by computerized tomography should be useful in man, if the method is to be applied clinically. An abstract of this study is in press (Neuwelt, E.A., Rapoport, S.I. et al., Abstracts Congress of Neurological Surgeons, Oct. 1979 (in press).

C. Pathological Effects of Blood-Brain Barrier Breakdown.

1. Blood-brain barrier integrity in senescent rats. A hypothesis for aging of the central nervous system is that the blood-brain barrier breaks down so as to allow potentially neurotoxic brain-reactive antibodies in plasma into brain. We tested this hypothesis in rats by measuring cerebrovascular permeability to ^{14}C-sucrose (see above) in 3-month and 28-month conscious Fisher 344 rats. Sucrose permeability was equal in both groups. As it normally is extremely low, 2 x 10^{-8} cm/sec, any change in permeability would be easily measured. The findings thus do not support the immune hypothesis for brain aging. We did find, however, suggestive evidence that the brain extracellular space was reduced by about 50% in aged animals, in accord with Bondareff's findings with freeze-substitution. A reduced space might limit intercellular diffusion within the aged brain (Ref. 8).

2. Threshold for hypertensive breakdown of blood-brain barrier. Autoregulation of cerebral blood flow is said to protect the cerebrovasculature from the deleterious effects of systemic hypertension. In order to examine the upper pressure limit of this protection, isotonic saline solution was infused for 15 sec into the common carotid artery of monkeys and rabbits, when the external carotid artery was clamped temporarily and the lingual was catheterized for measuring pressure. Opening of the blood-brain barrier was monitored by studying brain extravasation of intravascular Evans blue, a dye which binds to plasma protein and normally does not stain the brain. Barrier opening correlated better with infusion pressure than with infusion rate, and occurred at carotid artery pressures above 170 mm Hg. Systemic hypertension induced by Aramine increased barrier vulnerability by causing a higher net carotid artery pressure to be attained at a given infusion rate. The experiments provide an upper limit for protection of the blood-brain barrier against systemic hypertension (170 mm Hg), and suggest that carotid angiography in hypertensive individuals may lead to blood-brain barrier opening (Ref. 9).

3. Relation of cerebrovascular permeability to brain edema. The observation that reversible increases in cerebrovascular permeability produce a transient 2 hr to 24 hr brain edema (see above and ref. 4,5), was used to formulate a new theory of edema. A hydraulic model of the intracranial compartments was used to analyze the quantitative contribution of various factors to vasogenic brain edema. These include osmotic and hydrostatic pressures, tissue compliance and capillary and tissue hydraulic conductivities. The model predicts that edema will develop if there is an elevated driving force for filtration across the normal capillary bed, or if the capillary permeability and hydraulic conductivity are increased. In the former case, filtrate does not contain plasma salt, and therefore dilutes perivascular osmolality and reduces the driving force for further filtration. If the capillary becomes permeant to salt, dilutional protection is absent, brain swelling is greater and a function of interstitial hydrostatic pressure and regional brain compliance k. Vasogenic edema might be treated by reducing elevated capillary permeability and hydraulic conductivity, by reducing brain compliance or, if brain hydraulic conductivity also is elevated, by reducing cerebrospinal fluid pressure (Ref. 4,5,9).

Significance to Biomedical Research and to the Program of the Institute:

1. An understanding of how the blood-brain barrier regulates the brain environment and controls brain metabolism is critical to interpreting changes of brain function in health, aging and disease. Our findings establish the ultrastructural and transport properties of the blood-brain barrier.

2. Our methods in conscious rats, together with pharmacokinetic theories that we are developing, make it possible to measure cerebrovascular permeability of drugs as a function of their physical properties, and to measure

transfer constants between blood and brain compartments. It should be possible, on the basis of the findings, to predict and interpret dose-response relations of centrally acting drugs in man and animals. An altered response in relation to age could be due to an altered rate of drug entry into the brain or to changes in cerebral receptor sensitivity.

3. Our demonstration that opioid peptides have a significant cerebrovascular permeability, supports experiments that show that they have central effects when injected systemically, provided their plasma half-life is longer than several minutes. Feedback may operate between circulating peptides with potential central effects and brain sites that regulate their release into the systemic circulation.

4. Reversible osmotic opening of the blood-brain barrier is the first and only method that allows normally excluded agents into the brain without producing long-term brain damage. Because of its potential use in man, our studies that quantify threshold of infusate concentration and infusion duration for osmotic opening are critical, and our description of the time course of increased permeability provides a basis for determination of therapeutic regimens.

5. Our experiments and theory on brain water accumulation establish for the first time a quantitative comprehensive relation between critical factors that determine the appearance and time course of brain edema, and establish principles for treatment of this malady. Brain edema confounds trauma, infection and hypertensive brain disease, and contributes to the malignant course of these disturbances in man.

6. Experiments with blood-brain barrier opening in relation to carotid artery pressure provide an upper limit for protection of the barrier against systemic hypertension, and suggest that carotid angiography in hypertensive individuals may produce cerebrovascular damage.

7. As cerebrovascular integrity is not increased in aged rats, the hypothesis that entry of brain-reactive proteins into brain augments cell death must be reevaluated. The calculated decline in brain extracellular space with age suggests that intercellular diffusion is altered in the senile brain.

Proposed Course: (a) The pharmacokinetic rules that govern drug transfer from plasma to brain compartments will be elaborated, and models will be developed and tested to interpret dose-response relations of centrally acting drugs in animals and man, (b) the metabolism and pharmacokinetics of fluorazepam, a drug of clinical interest in aging, will be examined in detail, (c) the structure and function of the blood-brain barrier and the values for brain spaces will be examined quantitatively in relation to aging in the rat model, (d) sites and mechanisms for protein entry into cerebrospinal fluid will be analyzed, the kinetic properties of blood-brain barrier transport of critical substrates for brain metabolism will be analyzed, (f) methods will be examined to investigate permeability of barriers in the retina and other parts of the eye in rats, in relation to aging, (g) further ultrastructural studies will be performed to define the basis of increased cerebrovascular permeability following osmotic treatment, and (h) further methodology of barrier opening will be elaborated for the possible application of the method to clinical diseases such as metastatic brain tumor.

Publications:

1. Ohno, K., Pettigrew, K.D., and Rapoport, S.I.: Lower limits of cerebrovascular permeability to nonelectrolytes in the conscious rat. American Journal of Physiology: 235(3): H299-307, 1978.
2. Barranger, J.A., Rapoport, S.I., Fredericks, W.R., Pentchev, P.G., MacDermot, K.D., Steusing, J.K., and Brady, R.O.: Modification of the blood-brain barrier: Increased concentration and fate of enzymes entering the brain. Proceedings National Academy of Sciences 76: 481-485, 1979.
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4. Rapoport, S.I.: Roles of cerebrovascular permeability, brain compliance, and brain hydraulic conductivity in vasogenic brain edema. In Popp, A.J., Bourke, R.S., Nelson, L.R., and Kimelberg, H.K. (Eds.): Neural Trauma Seminars in Neurological Surgery. New York, Raven Press, 1979, pp. 51-62.
5. Rapoport, S.I.: A mathematical model for vasogenic brain edema. Journal of Theoretical Biology 74: 439-468, 1978.
6. Pappius, H.M., Savaki, H.E., Fieschi, C., Rapoport, S.I., and Sokoloff, L.: Osmotic opening of blood-brain barrier and local cerebral glucose utilization. Annals of Neurology 5: 211-219, 1979.
7. Rapoport, S.I., Ohno, K., Fredericks, W.R., and Pettigrew, K.D.: Regional cerebrovascular permeability to (^{14}C) sucrose after osmotic opening of the blood-brain barrier. Brain Research 150: 653-657, 1978.
8. Rapoport, S.I., Ohno, K., and Pettigrew, K.D.: Blood-brain barrier permeability in senescent rats. J. Gerontology 34: 162-169, 1979.
9. Rapoport, S.I.: Blood-brain barrier opening by isotonic saline infusion in normotensive and hypertensive animals. Acta Radiological Diagnosis 19: 921-932, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00121-02 LNS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Function of Peripheral Nerve and Muscle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	S.I. Rapoport	Chief	LNS	NIA
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	A. Weerasuriya	Visiting Fellow	LB	NINCDS

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A. Fatigue of single frog muscle fibers appeared as a decline in tension output during prolonged tetanic stimulation. The concentration of ATP (energy source for contraction) remained normal in fatigued fibers. Furthermore, ATP was not sequestered or unavailable for contraction. Fatigue therefore was due to an inability of the muscle to use energy in the form of ATP. We suggest that H^+, as lactic acid, accumulated in these fibers so as to interfere with excitation-contraction coupling. B. The perineurial sheath which surrounds peripheral nerve axons may regulate nerve function and development. Perineurial permeability was measured for the first time (to ^{14}C-sucrose) and was found to be very low, supporting a regulatory role for the sheath. Permeability was increased by stretch and by soaking in hypertonic solutions. Morphological studies showed that the perineurium can vary in number of cell layers, and that the most internal layers act as a barrier to lanthanum and horseradish peroxidase, due to tight junctions between the cells.

PHS-6040
(Rev. 10-76)

Project Description:

The object of this project is to relate metabolism and morphology of peripheral nerve and muscle to the functioning of these tissues in health, disease and aging.

Objectives:

A. Muscle contraction and muscle fatigue. When a striated muscle is stimulated repetitively, its contractile force decreases and it is said to be fatigued. Fatigue characterizes fast twitch muscles whose metabolism is glycolytic, rather than slow twitch muscles whose metabolism is oxidative. Muscle fatigue generally is thought to be caused by depletion of available energy reserves. However, some studies in single fibers suggest that fatigue can be due to uncoupling of steps that connect excitation with contraction. We explored the relation between fatigue and metabolism in single muscle fibers rather than in whole muscles, because, in whole muscles, some fibers can fatigue faster than others and measured metabolite concentrations are averaged over a heterogeneous, often disparate fiber population. Furthermore, intercellular diffusion limits physiological studies of whole muscles but not of single fibers.

B. Peripheral nerve and perineurium. Peripheral nerve function in health and disease may be governed in part by the local axonal milieu, which is separated from body fluids by a perineurial sheath and by endoneurial capillaries. We wanted to study the function and morphology of the perineurium, in order to understand the role of the perineurium in regulating and/or maintaining the endoneurial environment of axons, and in affecting peripheral nerve function.

The electrical properties of the perineurium were examined, as well as its ability to act as a permeability barrier to radiotracers (nonelectrolytes and ions) and electron-dense tracers (lanthanum and horseradish peroxidase). These properties were related to overall function and morphology of perineurial cells.

Methods Employed:

A. Muscle. A single fiber was dissected from the frog semitendinosus muscle and mounted in a bath of flowing Ringers solution at 15°C. Tension was recorded by a RCA 5734 transducer tied to one end of the fiber; the output was displayed on an oscilloscope face. Sarcomere length was measured by a laser diffraction technique. The fiber was stimulated at different frequencies *via* external platinum electrodes, so as to produce twitches or tetanic contractions for up to 200 sec.

B. Perineurium. For electrical and flux studies, the perineurium of the frog sciatic nerve was isolated and mounted on cannulae within a bath of stirred Ringers solution and perfused by Ringers solution which could be collected. Radioisotopes were placed in the bath. Transport through the sheath was quantified by measuring isotope entry into perfusate.

Electrical resistance and capacity of the sheath were measured with internal and external voltage-recording and current-supplying electrodes.

For morphological studies, the frog sciatic nerve was fixed with glutaraldehyde in situ and/or in vitro by immersion. More rapid preservation was achieved by using an osmium tetroxide-glutaraldehyde fixative. For tracer studies, colloidal lanthanum or lanthanum chloride was presented for 3 to 4 hours externally to small segments of nerve, or internally by injection. Modifications in the standard lanthanum procedure were done as controls for the effectiveness of lanthanum as a tracer. Horseradish peroxidase was used as an in vitro tracer in the bath for one hour at 25° or 5°C and reacted with 3,3-diamino-benzidine and hydrogen peroxide. Tissues were prepared for electron microscopic examination.

Major Findings:

A. Muscle. We first distinguished the time course of the fatigue process in single fibers from post-tetanic potentiation of the twitch, which is characterized by elevation and prolongation of the twitch and is due to intracellular calcium accumulation. Fatigue appeared after 20 sec of tetanic stimulation (at 20 Hz) as a decline in tetanic tension that took as long as 1 hr to be reversed. We then correlated the decline and recovery of tension with the instantaneous metabolic profile of the fiber. We found that prolonged tetanic stimulation reduced fiber phospho-creatine (PCr) in proportion to the internal work performed by the fiber, as measured by the isometric time-tension integral. However, despite the fact that ATP is the immediate energy source for muscle contraction, ATP concentration was normal or close to normal in fatigued fibers. ATP was not sequestered in internal compartments in these fibers, as residual ATP could be consumed when caffeine was applied to the fibers to produce maximal and irreversible contractures. Thus, fatigue was not due to depletion of available energy stores for muscle contraction, but to failure of coupling between excitation and contraction. On the basis of measured lactate and ATP/PCr ratios in fatigued fibers, we suggested that H^+, as lactic acid, accumulates in repetitively stimulated muscle and interferes with the action of calcium in activating muscle contraction. (Ref. 1).

B. Perineurium. (1) Electrical and flux studies. The permeability coefficient of ^{14}C-sucrose at the isolated perineurium equaled 5.6×10^{-6} cm/sec. This value is very low and shows that the sheath is extremely impermeable to water-soluble solutes. Permeability to ^{14}C-sucrose was increased reversibly by stretching the perineurium by 10%, but was increased irreversibly by further stretch or by immersing the sheath in a Ringers solution made hypertonic with either NaCl or sucrose. We suggested that stretch or hypertonicity modifies the dimensions of tight junctions that connect perineurial cells, and thereby increases permeability.

In order to examine the electrical and ionic permeability properties of the mounted and perfused perineurium of the frog sciatic nerve, an AC current was passed across the perineurial cylinder wall and voltage across the wall was measured. Frequency of current varied from 1 Hz to 0.1 MHz. The DC resistance of the perineurium was 430 ohm cm^2, and impedance measurements demonstrated two dispersions with center frequencies of 5 KHz and 20 Hz. Exposure to high conductance Ringers decreased the DC resistance; a low conductance Ringer increased the resistance. Analysis of the data in terms of a four-element model (two resistors and two capacitors) for an equivalent circuit suggested that small (0.1 $\mu F/cm^2$) and large (20 $\mu F/cm^2$) capacitances exist at the perineurium. The small capacitance indicates the presence of six or more layers of cells and was unchanged by experimental manipulations. The large capacitance could be ascribed to polarization of charge, perhaps at intercellular tight junctions, and was affected by experimental manipulation. An abstract of this has been published (Weerasuriya, A., R. Spangler, S.I. Rapoport and R.E. Taylor. Electrical impedance of the work perineurium of the frog sciatic nerve. Biophysical Journal 25, (No. 2, part 2) p. 300a, 1979).

The perineurial sodium permeability equaled 1.5 x 10^{-6} cm/sec and was unaffected by inhibitors of active transport. The permeability ratios of Na/K/Cl indicate that there is no discrimination between K and Cl and that the K/Na ratio is greater than the limiting conductance ratio in free solution. An abstract of this work has been published (Weerasuriya, A., Rapoport, S.I. and Taylor, R.E. Electrolyte permeability of the perineurium. The Physiologist, August, 1979).

(2) Morphological Studies. The frog sciatic perineurial sheath varied in its number of cell layers within a given level of nerve and varied from 7 to 19 layers among nerves of similar size. Close membrane appositions which probably represent tight junctions (zonulae occludentes), were seen between neighboring cells throughout the cell layers. The most external and internal layers were usually loose and incomplete. Unattached cell processes also were seen within the sheath. Lanthanum and horseradish peroxidase tracers penetrated from the external bath to varying depths in the perineurium. Most tracer remained in the outer layers and in all cases seemed unable to pass across the most internal, complete cell layer to the endoneurium. The very spotty appearance of lanthanum within the perineurium suggested that its penetration was limited to a very few pathways. Lanthanum was present in the endoneurium, within myelin and between Schwann cells and axons, only after its injection into the sciatic nerve. The narrow perineurial cells had numerous vesicles, most of which were fused to the external and internal cell membranes to form pits. In some rare cases, channels of lanthanum were seen to extend across the cells via fused vesicles.

Significance to Biomedical Research and the Program at the Institute:

A. Our finding that muscle fatigue develops, despite high levels of ATP corresponds to observations of "power failure" in stimulated peripheral nerve and sympathetic ganglion. In these tissues, function also fails before energy supplies are exhausted. Protection of energy supplies from exhaustion, may preserve survival of neuronal and muscle cells. Muscle wasting and fatigue commonly accompany senescence in man, but may be partially reversed, as endurance training can reduce fatigue despite age (Suominen, *et al.*, *J. Gerontol*. 32,33, 1977). Endurance training also increases muscle aerobic metabolism. If our hypothesis is correct, that lactate accumulation rather than energy exhaustion contributes to fatigue, then the results in man may reflect an enhanced ability of endurance-trained muscles to oxidize lactic acid.

B. Little is known about how the perineurium helps to regulate and maintain the metabolic and ionic environment of peripheral nerve axons. Defects in the perineurium may contribute to peripheral neuropathy in disease and aging. Basic information on the perineurium is required to address these questions.

Proposed Course:

(A) Energy consumption associated with the independent steps of excitation and contraction in single muscle fibers will be analyzed. Excitation can be dissociated from contraction by stretching fibers beyond the point where actin and myosin filaments overlap, or by placing fibers in solutions made hypertonic with sucrose.

(B) We will continue to investigate the functional morphology of the perineurium by directly correlating results seen with electron microscopy with physiological measurements of radiotracer permeability across isolated perineurial preparations, during different experimental manipulations. These studies will then be continued on the mammalian perineurial preparation and expanded to examine function and ultrastructure in peripheral neuropathy and during aging.

Publications:

1. Nassar-Gentina, V., Passonneau, J.V., Vergara, J.L., and Rapoport, S.I.: Metabolic correlates of fatigue and of recovery from fatigue in single frog muscle fibers. *Journal of General Physiology*, 72: 593-606, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00122-02 LNS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Pharmacology of Central and Peripheral Catecholaminergic Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	S.I. Rapoport	Chief	LNS	NIA

Others: None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project was to study the effect of aging, stress and antihypertensive drugs on the neuronal activities of both the central and peripheral catecholaminergic nervous systems and on the function of the cardiovascular system. (1) An age-dependent increase in blood pressure was found in spontaneously hypertensive, in Fisher-344 and in Wistar rats. (2) Immobilization stress induced increases in plasma catecholamines, corticosteroid and blood sugar that were not correlated significantly with age of animals. The aged group appeared to have a slowed rate of release of these substances into the circulation. (3) Stress produced an α-adrenergic vasopressor response in young rats but a β-adrenergic vasodepressor response in old rats. Over 90% of the old rats died within 12 hr after 30-min of immobilization stress. The stress-induced increase in heart rate was significantly reduced with age. (4) The age-dependent increase in blood pressure was effectively decreased by the administration of Uncarine A, the active ingredient of a Chinese herbal medicine. It reduced the blood pressure of hypertensive but not normotensive animals for up to 48 hr.

PHS-6040
(Rev. 10-76)

Objectives: (1) The present experiments were designed to investigate the cardiovascular reactivity of aged rats in response to 30 min of immobilization stress. (2) The project explores the relation among the cardiovascular, pituitary-adrenal cortical and sympathetic systems in freely moving rats. (3) The pharmacological action of Uncarine A, an alkaloid isolated from Uncaria Formasana, was investigated in order to provide scientific evidence for its usage as antihypertensive agent by Chinese barefoot doctors.

Methods Employed: (1) Chronic indwelling tail arterial catheter. One or two days before an experiment, an indwelling catheter filled with heparin-saline was inserted into the tail artery. The cannula was led subcutaneously up to and out at the back of the neck, where it was threaded through a spring wire which was anchored at the neck. The catheter was flushed twice daily with heparin saline. Rats were housed individually in a 30 x 30 x 8 cm plastic cage.

(2) Measurement of blood pressure and heart rate in conscious and unrestrained rats. Systolic and diastolic blood pressures of rats were monitored by a pressure transducer which was connected to the indwelling arterial catheter, and recorded polygraphically. The heart rates were triggered by the pulses of the blood pressure, and recorded by a polygraph.

(3) Assay for plasma catecholamines, corticosterone and blood sugar. Plasma samples (50 μl) were assayed for catecholamines by a radioenzymatic procedure. Corticosterone in 50 μl of plasma was measured by a spectrofluorometric procedure. Sugar in 10 μl of plasma was determined by an enzymatic procedure, using a Beckman glucose analyzer.

(4) Stress. Immobilization stress of rats was performed by tying down four limbs of the animals on a board.

Major findings:

(1) NIA Wistar Rats. During 30 min of immobilization stress, both systolic and diastolic blood pressure and heart rate were significantly increased in 3 month old Wistar rats. At 2 years of age, there was a decreased stress-induced increase in heart rate by 50%, and completely reversed stress-induced blood pressure changes. Stress decreased both the systolic and diastolic blood pressure by 20 mm Hg in 1 to 2 year old rats. 30 min after stress, the blood pressure of young rats returned to control levels, whereas the blood pressure of old rats was decreased further, approximately 30 to 40 mm Hg below control. The continued decine of blood pressure and heart rate in old rats after stress was associated with a significant increase in mortality rate, to 90% at 12 hours after stress. The sympathetic, adrenal cortical and medullary systems were not significantly altered by age. Slight decreases in plasma epinephrine and blood sugar after stress were observed in old rats. The age-induced reversal of cardiovascular responses during and after stress is not due to a lack of sympathoadrenal responsiveness.

(2) Fisher-344 Rats. The age-related changes in cardiovascular responsiveness to stress were not similar to those of Wistar rats. The mortality of young (3-month old) was greater than adult (12-month old) and less than of old (28-month old) rats. The 12-month old Fisher rats' responsiveness was equivalent to that of 3-month old Wistar rats. During 30 min of immobilization stress, the increases in blood pressure and heart rate were higher than in other age groups. After stress, the blood pressure and heart rate of old and young, but not of adult rats were decreased below control. In general, the plasma catecholamines and corticosterone were the lowest in the high survival group while the increases in heart rate and blood pressure were the highest. Thus, immobilization seems less stressful to 3-month old Wistar and 12-month old Fisher-344 rats while their cardiovascular system maintains adequate blood pressure for survival. The results of adrenergic and cardiovascular responsiveness of 28-month old Fisher-344 rats following stress will be presented at the 1979 Society of Neuroscience Annual Meeting (Abstract #97.27) in Atlanta, Georgia. (Adrenergic and cardiovascular responsiveness of 28-month old Fisher-344 rats following stress by C.C. Chiueh and S.I. Rapoport).

(3) Uncarine A. Uncarine A, 2 to 40 mg/kg, produced a long-lasting (24-48 hr) hypotensive action in hypertensive animals, but it had little effect on the normotensive rat. The mode of action of Uncarine A is not similar to those of the current antihypertensive agents, i.e., clonidine, reserpine, α and β receptor blocking drugs. A preliminary report was published in Neuroscience Abstract 4:18 (Abstract #49), 1978. (Effects of Uncarine A, clonidine and 5-(4'-chloro) butylpicolinic acid (FD008) on blood pressure and sympathetic activity in unanesthetized spontaneously hypertensive rats by C.C. Chiueh, S.M. Nespor and C.C. Chang).

Significance to Biomedical Research and to the Program of the Institute:

(1) The results demonstrate resting cardiovascular, pituitary adrenal-cortical and sympathoadrenal activities, in conscious and freely moving Wistar rats (24-month life span) and Fisher-344 rats (36-month life span). At 12-months of age, Wistar rats reverse their stress-induced vasopressor responses into vasodepressor responses, whereas Fisher-344 rats retain the stress-induced increase in cardiovascular responsiveness that appears essential for survival after stress. An age difference in the maintenance of cardiovascular reactivity to stress may play an important role in longevity of rodents and of humans.

(2) The study of Uncarine A may provide the pharmacological basis for use of oxindole alkaloids for the clinical treatment of essential hypertension. The duration of the hypertensive action of Uncarine A makes it more convenient than current drugs, for the chronic management of high blood pressure in the elderly.

Proposed Course:

(1) Physiological and pharmacological coping with stress in the elderly.

The possibility of the involvement of a myocardial depressant factor or the alteration of α-adrenergic (vasopressor) to β-adrenergic (vasodepressor receptor activity in stressed old rats will be investigated in order to find a physiological or pharmacological means to improve survival rate and cardiovascular reactivity after stress.

(2) Structure and activity relationship of Uncarine A. Because of the difficulty in obtaining Uncarine A alkaloids, this compound will be synthesized by chemical procedures. The structure-activity relation will be investigated in synthesized analogues of oxindole, in order to find a safe and new antihypertensive drug.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00123-01 LNS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Synapse Development, Specificity and Mechanism in Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.M. Thompson	Staff Fellow	LNS	NIA
Others:	S.I. Rapoport	Chief	LNS	NIA
	M. Nirenberg	Chief	LBG	NHLBI
	R.R. Ruffolo, Jr.	PRAT Fellow	LBG	NHLBI
	G.S. Eisenbarth	Clinical Associate	LBG	NHLBI
	G.L. Cantoni	Chief	LGCB	NIMH
	P.R. Chiang	Sr Staff Fellow	LGCB	NIMH

COOPERATING UNITS (if any)

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Laboratory of General and Comparative Biochemistry, NIMH

LAB/BRANCH
Gerontology Research Center, Laboratory of Neurosciences

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NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Synapse formation, synapse termination, synapse specificity and neurotransmission were studied using neurons and muscle cells in culture. Synapses were detected and investigated by electrophysiological recording. Neurons from chick and rat retina form transient synapses with muscle cells, whereas neurons from chick spinal cord form long-lived synapses with muscle cells. Each type of neuron possesses a synapse-competent state in its development during which it can abundantly form synapses with muscle cells in culture. These results suggest a mechanism of synapse specificity based on stabilization of correct synapses coupled with a synaptogenic period during development for each type of neuron. In addition, it appears likely that a methyltransferase reaction is involved in neurotransmission.

GRC/LNS-192

Objectives: Neurons in culture form synapses with other neurons and/or muscle cells as shown by electrophysiological recording and electron microscopy. The types of neurons, their target cells and the culture environment can be manipulated in order to study a) neurotransmission, b) synapse formation, c) synapse termination and d) synapse specificity. This system allows one to study how development, aging, drugs and disease affect the above properties of neuronal connections.

Methods Employed: Striated muscle cells isolated from newborn rats grow in culture, form myotubes, develop nicotinic acetylcholine receptors and become electrically active. Neurons from chick, mouse or rat spinal cord and retina, and from neuroblastoma cell lines form synapses with these muscle cells in culture. Synapses are detected by electrophysiological recording. In addition, biochemical measurements can be performed on the cultured cells.

Major Findings: Synapse development. Neurons from chick embryo retina form synapses with muscle cells in culture during a discrete period in their development. In addition, the synapses formed are transient and disappear with a half-time of 21 hr (Ruffolo, Eisenbarth, Thompson and Nirenberg, 1978, Proc. Natl. Acad. Sci., U.S.A. 75:2281-2285; Puro et al., 1977, Proc. Natl. Acad. Sci., U.S.A. 74:4977-4981). Neurons from rat retina also form transient synapses with muscle cells in culture during a discrete period in development during which they form synapses and show a decline in the number of synapses formed, but do not completely lose the ability to form synapses. In addition, spinal cord neurons form synapses with muscle which do not disappear (Nirenberg, Wilson, Higashida, Rotter, Ray, Adler, Thompson and DeBlas, Fed. Proc, 38, 626, 1979).

The discrete developmental period for synapse formation may be a phenomenon which occurs for most neurons. Older neurons may therefore not be able to form any synapses or may only form relatively few synapses. It appears that inappropriate synapses are lost (e.g., retina-muscle synapses) while appropriate synapses are retained (e.g., spinal cord-muscle synapses). These results suggest that synapse specificity is imparted, not by specific formation of correct synapses, but by specific stabilization of correct synapses. In addition, the developmental stage of neurons and the length of their synapse-competent state may restrict their synaptic targets to cells which have reached the developmental period in which they are receptive to forming synapses.

Mechanism of neurotransmission. Methyltransferases can be inhibited by S-adenosyl homocysteine (AdoHcy) or by analogues of this compound. Several analogues of AdoHcy can increase the levels of AdoHcy by inhibiting S-adenosyl homocysteine hydrolase (Chiang, Richards and Cantoni, 1977, Mol. Pharmacol. 13:939-947; Chiang et al., 1978, Biochem. Biophys. Res. Commun. 82:417-423). When cultures of 8-day chick embryo neural retina cells and muscle cells were treated for 2 hr with these AdoHcy analogues, the number of muscle responses/ min in these synapses was inhibited. The inhibition was rapid and reversible. The results strongly suggest that a methyltransferase mediated reaction occurs during neurotransmission (Thompson, Nirenberg, Chiang and Cantoni, manuscript in preparation).

Significance to Biomedical Research and to the Program of the Institute:

The finding that neurons can abundantly form synapses only during a discrete period in their development may play an important role in synapse plasticity, regeneration and learning and memory in both young and aged organisms. An understanding of the mechanism of synapse specificity is important for studies of synapse formation and regeneration.

The discovery of the involvement of a methyltransferase in neurotransmission leads to a better understanding of how neurons communicate and how that communication can be changed.

Proposed Course: The model system of neurons in culture will be used to study: a) pharmacology of synapses and synaptic circuits, b) differences between transient and long-lived synapses; c) control of synapse-competent states and d) a locus of methyltransferase actions on neurotransmission.

Publications:

Thompson, J.M., Nickels, J.S. and Fisher, J.R.: Synthesis and degradation of xanthine dehydrogenase in chick liver: In vivo and in vitro studies. Biochimica et Biophysica Acta 568: 157-176, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00124-01 LNS

PERIOD COVERED
October 1, 1979 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Central Nervous System Binding Sites for the Neurotoxin Kainic Acid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.D. London	Staff Fellow	LNS NIA

Others: None

COOPERATING UNITS (if any)
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H.I. Yamamura, Prof, Dept Pharmacol, Univ of Arizona Hlth Sci Ctr, Tucson, AZ
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Binding of $[^3H]$ kainic acid ($[^3H]$KA) to neural sites was studied in invertebrates and vertebrates. Maximal binding is noted in brains of the frog, spiny dogfish, goldfish and chick. Significant binding is observed in some of the lowest forms. Generally, binding is to both high (K_D = 3-14nM) and low (K_D = 25-50nM) affinity receptors. In non-mammalian chordates, binding occurs in the cerebellum, with less in forebrain and least in the medulla. In mammals, maximal binding occurs in forebrain, with less in the cerebellum, and least in the medulla. Frog brain, rat forebrain and human parietal cortex show similar binding kinetics. In these vertebrates, unlabeled kainic acid (KA) is the most potent inhibitor of $[^3H]$ KA binding. Reduction of the isopropylene side chain of KA reduces potency. L-glutamate is less potent than KA; D-glutamate is less potent than L-glutamate. The widespread distribution of highly specific $[^3H]$KA binding sites in neural tissue and the similar binding characteristics of these sites in divergent vertebrate species suggest the presence of an endogenous neuronal system that has not changed appreciably through evolution.

PROJECT DESCRIPTION:

Objectives: Intrastriatal injection of the neurotoxin kainic acid (KA) to rats has provided an animal model for the human neurodegenerative disorder Huntington's disease (HD). Subtle modifications in the structure of KA eliminate its neurotoxic effects, indicating a high selectivity in the recognition sites mediating kainate's action. The marked potency of KA as a depolarizing agent indicates that these sites have a high affinity for the molecule. Accordingly, the specific binding of ^{3}H KA to neural membranes has been studied. The aims of this project are as follows: (a) to characterize possible receptor recognition sites that mediate KA neurotoxicity, (b) to determine the phylogenetic distribution of these sites in neural tissue, (c) to explore possible cooperative effects between KA and glutamic acid at the receptor level, and (d) to determine if there is a selective loss in ^{3}H KA binding sites in postmortem brains of HD patients.

Methods Employed:

(A) Preparation of washed membranes. Tissue for assay is taken from hydra, sea anemone, planaria, earthworm, crayfish, cockroach, goldfish, frog, hagfish, spiny dogfish, chicks, rat and human postmortem brain. Samples are sonified in glass distilled water, and the suspensions centrifuged at 48,000 x g for 10 min. at 2°. The membrane pellets are resuspended, pelleted and washed sequentially in 100 vol. glass distilled water and 50mM Tris-citrate buffer, pH 7.1. Finally, the membranes are suspended in 100 vol of 100 mM Tris-citrate buffer, pH 7.1. Protein is assayed by the method of Lowry *et al*. (1951).

(B) Standard ^{3}H Kainic Acid Binding Assay. 1000 1 of washed membrane suspension and various concentrations of radioligand displacers are placed in 15 ml Sorvall teflon centrifuge tubes. Ten to 300 pmoles of freshly diluted ^{3}H KA are added in a volume of 100 1 to start the reaction. The incubation volume is adjusted to 2 ml with distilled water. Incubations are run for 60 min at 2° and are stopped by centrifugation. Bound radioactivity in pellets is estimated by liquid scintillation spectroscopy. Nonspecific binding is determined in the presence of 0.1 mM unlabeled KA.

To characterize separately binding to high and low affinity sites, advantage is taken of the fact that the radioligand dissociates slowly from high affinity sites but nearly instantaneously from low affinity sites in rat and human brain. After 60 min of incubation with ^{3}H KA, 200 nmoles of unlabeled KA are added to some tubes, bringing the final concentration to 0.1 mM. After another 5 min of incubation, the samples are centrifuged and bound radioactivity measured. The addition of unlabeled ligand 5 min prior to centrifugation displaces radiogand from low affinity binding sites but does not affect nonspecific or high affinity binding.

MAJOR FINDINGS

(1) Densities of ^{3}H KA binding sites in various species. The highest densities of sites are noted in whole brains of the frog (94,100 fmol/mg protein) and goldfish (6560 fmol/mg protein) and brain regions of the spiny dogfish (6910-77,200 fmol/mg protein). Considerable binding is also noted

in brain regions of the smooth dogfish (455-2540 fmol/mg protein) and chick (356-2990 fmol/mg protein).

In other species, values vary inconsistently throughout the animal kingdom. While the hydra, one of the phylogenetically lowest forms, has considerable binding, the sea anemone has no sites detected. The nervous system of the earthworm, the rat brain and the cockroach brain have similar intermediate values for total specific binding. Overall, human brain has less specific binding in various regions than rat brain. This difference is most pronounced (3-fold) in forebrain structures.

2. Regional distribution of $[^3H]$KA binding sites in brain. In the non-mammalian species tested, the greatest density of binding sites is in the cerebellum with less in the midbrain and least in the medulla. In the two mammalian species, the highest density is in forebrain gray matter, with the most high affinity sites in the human caudate nucleus and putamen and the rat caudato-putamen. In rat cerebellum, binding is lower than in forebrain, while in man, cerebellar membranes have binding equivalent to that in parietal cortex. In rat and man, midbrain has intermediate levels of binding with low levels of binding in white matter.

In frog whole brain, rat forebrain and human parietal cortex, we observe two components of binding: KD_1 = 24-30 nM, KD_2 = 5-14 nM. In chick, rat and human cerebellum, there is only one binding site with a KD of 25-30 nM. In dogfish cerebellum, binding is to lower affinity sites than in forebrain.

The IC_{50} values in inhibition studies indicate that in frog brain, rat forebrain, and the human caudate nucleus and cerebellum, unlabeled KA is the most potent inhibitor of $[^3H]$KA binding. L-Glutamic acid is 20-180 fold less potent than KA; D-glutamic acid is 27-2540 fold less potent than the L-isomer; dihyrokanic acid is 115- to 30,000 fold less potent than KA, but almost equipotent to L-glutamic acid in human brain.

While inhibition of $[^3H]$KA by unlabeled KA follows simple mass action kinetics (Hill coefficient = 1.0), inhibition with glutamic acid or dihydrokainic acid acid shows anomalous kinetics with Hill coefficients approximating 0.5. These observations suggest that KA binds to a different receptor than L- and D-glutamic acids and dihydrokainic acid, and that these substances may interact cooperatively with KA at the receptor level. Portions of this work have been presented at the NINCDS Symposium of Huntington's Disease, 1978, San Diego, California (Coyle, J.T., London, E.D., Biziere, K. and Zaczek, R., 1978, Kainic acid neurotoxicity: Insights into the pathophysiology of Huntington's disease) and will be presented at the 1979 fall meeting of the Society for Neuroscience (London, E.D., Klemm, N., and Coyle, J.T., 1979, Phylogenetic distribution of $[^3H]$kainic acid receptor binding sites in neuronal tissue. Soc. for Neurosci. Abstracts).

Significance to Biomedical Research and to the Program of the Institute

We have demonstrated and characterized highly specific binding of [^{3}H]KA to receptor sites in neural tissue from various animal species. Binding characteristics, including affinities in the nanomolar range, are consistent with binding to neurotransmitter receptors. The wide distribution of binding sites in the animal kingdom and the remarkable similarity in binding characteristics across species lines supports the concept that [^{3}H]KA simulates an endogenous substance which binds to neural receptors. Our observation that, of the species tested, the frog brain has the highest density of [^{3}H]KA binding sites indicates that frog brain may be a good source of receptor for purification and further studies.

The demonstration of [^{3}H]KA binding sites in human brain with kinetic characteristics similar to those in the rat model for Huntington's disease (HD) indicate that kainate's neurotoxicity may be relevant to human disease. Notably, the caudate nucleus and putamen, which are severely affected in HD exhibit the highest densities of high affinity KA binding sites in human brain.

The cooperative effects noted between L-glutamate and KA at the receptor level, taken with the observation that KA is not toxic when glutamatergic innervation is removed, indicate that at the receptor level KA sensitizes neurons to the neurotoxic potential of glutamate-induced depolarization. Such an action of an endogenous brain substance in overabundance might be responsible for the degeneration seen in HD and other human disorders. Alternatively, an abnormality in membrane structure may render neurons more sensitive to the neurotoxic action of an endogenous substance, as KA renders striatal neurons sensitive to their afferent input.

Recently, local injections of KA have been used to selectively reduce the cholinergic innervation of the cortex in rats, resembling the major reported deficit in neurochemical senile dementia of the Alzheimer's type. Thus, the information obtained in these studies seem relevant not only to HD, but to several human diseases where specific cell loss may be linked with a membrane receptor-mediated phenomenon.

Proposed Course: Regions of control and Huntingtonian postmortem brains have been obtained from Dr. H.I. Yamamura, University of Arizona Health Science Center, Tucson, AZ. These samples are from age-matched subjects and have been coded. Dr. London will assay these samples for high and low affinity [^{3}H]KA binding sites as well as the activity of choline acetyltransferase.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00125-01 LNS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Cerebral Metabolism, Relation to Brain Function and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.D. London	Staff Fellow	LNS	NIA
	S.I. Rapoport	Chief	LNS	NIA
	D. Dow-Edwards	Staff Fellow	LNS	NIA
Other:	S. Nespor	Biol Lab Techn	LNS	NIA
	W. Fredericks	Biologist	LNS	NIA
	K. Ohno	Visiting Fellow	LNS	NIA
	L. Sokoloff	Chief	LCM	NIMH
	C. Smith	Staff Fellow	LCM	NIMH
	M. Shinohara	Visiting Scientist	LCM	NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1.1	1.1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

(1) The ^{14}C-2-deoxy-D-glucose method (Sokoloff et al., 1977) was used to study regional cerebral glucose metabolism as a function of age in Fisher 344 rats (3, 12 and 24 mo. animals). Structures which showed no significant decrements with age were cerebellar gray and white matter, nucleus accumbens, septum, hypothalamus and thalamus. Structures which showed reductions, of the order of 20-30%, were superior colliculus, inferior colliculus, medulla, midbrain, caudate nucleus, frontal and lateral cortex. Most changes occurred between 3 and 12 months of age rather than between 12 and 24 months, and probably reflect age-related altered functional states of the affected brain regions. (2) Cerebral glucose utilization in rats markedly increased in most areas of the cerebral cortex and declined in many subcortical structures during spreading cortical depression. Cortical glucose utilization remained elevated during recovery, and was distributed in perpendicular cortical columns. (3) ^{14}C-iodoantipyrine provided better measures of regional cerebral blood flow than did ^{14}C-antipyrine in conscious rats. ^{3}H-nicotine also could be employed to measure regional cerebral blood flow accurately.

GRC/LNS-199

Project Description.

Objectives: Numerous neurochemical, neuropathologic and behavioral changes during aging have been described in the rat as well as in man. The aims of this project are as follows: (1) to determine which, if any, specific areas of brain show selective alterations in glucose utilization, (2) to study cerebral glucose utilization in relation to heightened brain activity during spreading cortical depression and (3) to establish methods for measuring regional cerebral blood flow, in relation to activity and glucose utilization.

Methods Employed: Rats are lightly anesthetized with ether, implanted with venous and arterial femoral catheters, and their hindquarters restrained with a loose-fitting plaster cast taped to a wood block. Animals recover after 4 hours in a wood box, which is lined with sound absorbent material and equipped with a heating element. ^{14}C-2-deoxy-D-glucose (^{14}C-2-DG) (125 μCi/kg) is injected i.v., and timed arterial blood samples are collected over the next 45 min. These samples are assayed for glucose and ^{14}C-2-DG concentration. At 45 min, rats are killed with an i.v. overdose of sodium pentobarbital. Brains are rapidly removed and dissected into regions. The regions are weighed, solubilized, and the radioactivity assayed by liquid scintillation spectroscopy. The tissue ^{14}C-2-DG is estimated from the time course of the levels of ^{14}C-2-DG and glucose in plasma.

The physiologic state of the animal is assessed by monitoring blood gases and pH, heart rate, blood pressure and in some cases blood ketone bodies and catecholamines.

For studies of regional cerebral blood flow, conscious, constrained rats were infused i.v. with either ^{14}C-iodoantipyrine, ^{14}C-antipyrine or ^{3}H-nicotine, and decapitated from 30 sec to 4 min later. Brain and plasma radioactivity data were analyzed to calculate regional cerebral blood flows.

Spreading cortical depression was initiated in anesthetized rats by applying potassium-rich Ringers solution to the cerebral cortex, via a trephine hole in the skull. The process was followed by EEG recording.

Major Findings:

1. An initial study, presented in abstract form (Smith, C.B., Fredericks, W.R., Rapoport, S.I., and Sokoloff, L. Trans. Amer. Soc. Neurochem. 10; 171, 1979) demonstrated that local cerebral glucose consumption decreased significantly, in conscious Sprague-Dawley rats, between 3 and 12 months of age, and that deceases at 24 months of age were smaller and less statistically significant. One striking finding was a decreased consumption in cortical layer IV.

In Fisher 344 rats, baseline values for cerebral glucose consumption are somewhat less than those previously reported. This is probably attributed to the modifications we introduced into the method, specifically, the sound-insulated box and the long post-operative recovery period.

Cerebral glucose utilization is reduced 20-30% in most areas tested when 24 month old rats are compared with 3 month old rats, as shown in Table 1. Notable exceptions are the pituitary gland, the olfactory bulb and tubercle, the nucleus accumbens, septum and cerebellar gray and white matter. The major reductions in glucose utilization occur during the first year. Mean values ± S.E.M. for 3 rats per group are shown.

2. We also demonstrated that cerebral glucose utilization is markedly increased in most areas of the cerebral cortex and is reduced in many sub-cortical structures during spreading cortical depression, using the Sokoloff procedure. Spreading depression, a phenomenon first described by Leão in 1944, is a wave of cortical depolarization that moves along the cortex at a rate of 2-5 mm/min, as fast as the Jacksonian march of convulsions or the cortical movement predicted by migraine scotomata in man. During recovery from spreading cortical depression, cortical glucose utilization remains elevated, but the increased metabolic activity is distributed in columns running perpendicularly through the cortex (Ref. 1).

3. Regional cerebral blood flow in the conscious rat. Regional cerebral blood flow is often tightly coupled to regional cerebral metabolism, and as such is a measure of normal and abnormal brain function. It was measured by one of three methods in the conscious rat. Our findings confirmed those reported by Sakurada et al., (1978) that ^{14}C-iodoantipyrine provides better estimates of flow, approaching those obtained with an inert gas, than does ^{14}C-antipyrine. Furthermore, we demonstrated for the first time that ^{3}H-nicotine could be used as well to measure regional cerebral blood flow, and that its binding to brain tissue contributed to complete extraction in a single pass through the brain. We determined blood flow in dissected brain regions. Dissection allows direct determination of brain radioactivity and makes the measurement of flow simpler than with autoradiography (Ref. 2).

Significance to Biomedical Research and the Program at the Institute:

1. A knowledge of which brain regions have altered metabolism in the aging animal can help explain the altered behavioral function reported in aging.

2. By locating specific regions which have altered metabolism, we can direct future research to identifying which particular neurotransmitter or neuromodulatory systems are defective in these areas. Such identification could suggest appropriate pharmacotherapy to alleviate geriatric problems in cognition, perception and motor function.

3. Information about regional metabolism and blood flow in spreading cortical depression allows us to quantify cerebral metabolic demands during convulsive-like states, and to understand what factors contribute to increased brain activity.

4. Regional cerebral blood flow is often coupled directly to glucose consumption, and as such is a good measure of altered neuronal activity. Uncoupling occurs following damage to the blood-brain barrier and following edema, and may reflect rate-limitations in delivery of glucose and oxygen to the brain, leading to cell death. Blood flow can be measured in man in relation to brain pathology and aging. Animal studies help us to design clinical experiments and interpret human data.

Proposed Course: The work on age-related changes in cerebral glucose consumption will be continued and prepared for publication. Sample size will be increased and age range extended to 36 months. Glucose consumption of the visual pathway will be examined in detail. Glucose consumption results will be correlated with studies of regional cerebral blood flow, using ^{14}C-iodoantipyrine.

The effect of immobilization stress in rats will be investigated using blood flow and glucose consumption methods.

Publications:

1. Shinohara, M., Dollinger, B., Brown, G., Rapoport, S.I., and Sokoloff, L.: Changes in local cerebral glucose utilization during and following recovery from spreading cortical depression. Science 203: 188-190, 1979.

2. Ohno, K., Pettigrew, K.D., and Rapoport, S.I.: Local regional cerebral blood flow in the conscious rat, as measured with ^{14}C-antipyrine, ^{14}C-iodoantipyrine and ^{3}H-nicotine. Stroke 10: 62-67, 1979.

Table 1

REGION	GLUCOSE UTILIZATION (μmoles/100 g/min)		
	3 months	12 months	24 months
Pituitary gland	15.5 ± 2.8	27.4 ± 3.8*	17.1 ± 0.9
Frontal pole	66.8 ± 8.0	57.3 ± 5.0	55.5 ± 3.0
Olfactory bulb	56.3 ± 6.1	54.5 ± 2.8	52.5 ± 2.8
Olfactory tubercle	56.9 ± 7.0	50.4 ± 2.1	49.4 ± 3.3
Sensorimotor cortex	64.5 ± 5.3	55.3 ± 4.5	54.2 ± 3.6
Tempero-parieto-occipital cortex	62.0 ± 7.1	55.7 ± 2.6	47.6 ± 2.8
Head of caudate nucleus	64.6 ± 5.8	49.6 ± 6.6	46.2 ± 2.6*
Tail of caudate nucleus	50.8 ± 4.0	41.5 ± 7.7	40.5 ± 7.7
Nucleus accumbens	45.0 ± 6.6	47.4 ± 5.6	48.6 ± 8.5
Septum	44.6 ± 7.8	48.1 ± 7.1	44.4 ± 3.0
Hypothalamus & thalamus	43.8 ± 6.3	46.4 ± 8.1	39.5 ± 1.8
Midbrain less colliculi	46.9 ± 4.6	38.1 ± 4.6	34.3 ± 1.9
Inferior colliculus	82.6 ± 7.8	58.6 ± 3.4*	54.5 ± 2.6*
Superior colliculus	56.2 ± 4.6	49.1 ± 6.5	43.7 ± 2.4
Hippocampus	49.3 ± 3.7	41.9 ± 6.4	39.9 ± 2.3
Medulla	40.8 ± 4.0	29.7 ± 2.7*	26.6 ± 2.1*
Pons	42.5 ± 2.9	30.5 ± 3.2*	28.0 ± 1.8*
Cerebellar gray	39.6 ± 4.3	39.0 ± 6.7	39.1 ± 9.9
Corpus callosum	31.0 ± 6.1	18.9 ± 5.5	21.2 ± 4.8
Optic nerve	20.4 ± 6.6	17.9 ± 1.9	16.8 ± 4.0
Cerebellar white	38.3 ± 5.5	37.2 ± 3.9	37.8 ± 3.6

A preliminary report of this work will be presented at the September 1979 meeting of the American Aging Association.

* Statistically different from 3 month results ($p < 0.05$).

N = 3

Clinical Physiology Branch

The Baltimore Longitudinal Study of Aging has continued to maintain its male population and to increase its female population. There are now 643 active male participants and 162 women. Of the 1108 men who were seen at least once 200 have died and 265 have withdrawn. In the past year, an intensive tracing effort has been made to update information on the "lost" men. This was most successful--we have been able to trace virtually all participants and many have become "active" again. The growth of the female study is both heartening and disappointing. The success of the Illness and Disability follow-up study of the men in getting them to re-enlist decreased the number of new participants (primarily women) who could be brought in with our essentially fixed staffing pattern. Outside the CPB, the addition of a Stress and Coping Section has greatly extended the potentialities for study of those neglected variables as well as of personality factors in our subjects. The establishment of a Laboratory of Neurosciences will also in time have an important influence in the continuing evolution of the BLSA.

The Immunology Section has continued to assess the immunological potential of the subjects in the BLSA. During this second year of these studies, 210 individuals, of which 163 were women introduced for the first time, were tested using a wide variety of assays. With a total of 373 subjects now screened, the outstanding age-related change is associated with a decrease in T-lymphocyte proliferative responses to mitogens. This decrease in responsiveness is not correlated with changes in levels of peripheral blood white cells, lymphocytes T-rosetting cells, or naturally occurring killer cells. These cellular levels as well as levels of granulocytes, monocytes, and surface immunoglobulin positive cells remain strkingly constant among the different age groups. The activation of immunoglobulin secreting cells by pokeweed also does not change with advancing age. This correlates well with the constant levels of serum immunoproteins observed in the various age groups.

Studies directly assessing the host-parasite relationship in the subjects in the BLSA has been undertaken. The older subjects in the longitudinal population have significantly higher levels of antipneumococcal antibody than the younger subjects. The significance of these elevated titers must be viewed in light of an experimental model recently introduced. Mice of various ages (6, 12, 18, 24 and 30 months) were immunized with Pneumococcal antigens, serum titers determined and then challenged with viable Pneumococcus. Although the titers of serum antibody were not significantly different in the various age groups, the older mice suffers a considerably high lethality. This apparently is a result of a defect in the ability of cells from the old mice to kill the bacteria. As a result, a micro system of assessing bacterial killing by human peripheral blood granulocytes was devised and replaces the metabolic assay previously used. Studies are now in progress to determine if older humans suffer a similar granulocytic defect and if high levels of antibodies to Pneumococcus correlates with the killing ability of granulocytes.

Since 31 of the male subjeects examined this year had previously been examined, the reliability and reproducibility of the various assays can be established. Some of these assays may be discontinued or replaced and attempts to develop new assays to assess the immunological potential of humans will continue.

Studies using the murine models are centered around the cellular requirements and interactions involved in the induction of the immune response as well as the characteristics of cells carrying out the various immunological functions. It is now clear that the role of macrophages in the *in vitro* immune response can be completely substituted for by a soluble factor derived from macrophages. This factor appears to require the presence of T-lymphocytes to exert its effect on B-lymphocytes. This information was applied to studies on aging mice to establish any cellular deficiencies which occur as a result of advancing age. Deficiencies in each one of the cell types (macrophages, T-cells and B-cells) occur in older mice but there is no obvious chronological order of the cellular dysfunctions. Attempts to re-establish the activity of one of the cell types, namely thymus-derived lymphocytes, by administration of thymopoietin has not been successful.

The experimental murine model was also used to study the cell-mediated immune responses concerned with both induced and naturally occurring killer cells. *In vivo* studies show that immunological memory in the induced cytotoxic system is relatively short-lived. This may be due to the observation that during the *in vitro* development of induced cytotoxic cells, a population of suppressor cells has been shown to occur. These suppressor cells are able to ablate the development of any newly arising killer cells. The importance of this regulating cell type is being investigated to determine if such cells increase in aging mice and play a role in the observed reduction of cytotoxic cells as a function of age.

Naturally occurring killer cells in mice are lymphoid in nature but clearly represent a different cell type than the induced killer cells. Studies have shown that this natural killer cell is a non T or B lymphocyte. The activity of these cells is strain and age dependent and offers a means of measuring age-related changes in this subpopulation of lymphocytes. Another newly introduced assay is the lymphocyte adherence inhibition assay. This assay which is also most likely a cell-mediated form of immunity has been refined and is very reproducible. Both of these assays make use of tumor cells as targets and will be used to evaluate the responses of aging mice to various tumors.

Murine splenic lymphocytes from young and 15 month old mice were cryopreserved using the microprocessor-controlled freezing system developed in this laboratory. This freezing system, in contrast to others, has reduced selective injury to subpopulations of lymphocytes from young mice cooled over a wide range of rates and is desirable for use in the detection of possible age-related damage to cryopreserved cells. After cryopreservation at optimal cooling rates, the recoveries of viable cells were about 25% lower in cell suspensions of 15 month old mice than in those of young mice. There was selective damage to the T-lymphocyte subpopulations of the older mice, as shown by in vitro responses to mitogens which assess their functional capacity. Since the cells tested were viable, this suggests that there were discrete changes in cellular structure and/or function with age and that these changes were reflected in the impaired restoration of function after cryopreservation.

The Endocrinology Section has continued its work on the identification and elucidation of age-related alterations of hormone action. Age-related changes of cell surface and intracellular hormone receptors continue to receive attention. Attempts are being made to relate receptor changes to those of physiologic function. For example, aging in the rat has been reported to be associated with diminished responsiveness to β-receptor catecholamine (epinephrine) sensitive lipolysis. A study of isolated fat cells has been performed in which the mechanism of this change has been explored. Simultaneous measurements were made of the number of β-receptors, activation of adenylate cyclase, and the generation of intracellular cAMP. Lipolysis was age dependent with increased sensitivity in the senescent animals. Although the number and affinity of β-receptors and the activation of adenylate cyclase were unchanged, intracellular cAMP concentration was much reduced in the old animals. These findings suggest that an explanation of altered hormone responsiveness in the fat cell will require examination of variables other than those in the adenylate cyclase system. The results are also different from those seen elsewhere, suggesting the possible interaction of age and other factors in control of hormone-sensitive lipolysis.

Continued efforts are being made to elucidate the mechanism of the age-related decrease in function seen in the Leydig cells of the testis. Over the past year efforts have focused on the reversal of these changes with pharmacologic doses of gonadotrophin. Work has also continued on the relationship of human aging to gonadal and sexual function in collaboration with the Human Performance Section.

In the rat, reactivation of aged Leydig cells occurs despite a gonadotrophin mediated loss of membrane receptors which is slightly greater on a percentage basis than that seen in younger cells. Human work has shown a surprising lack of change in steroid sex hormones in BLS men, a result which stands in contrast to what has been reported by others. Loss of sexual activity appears to be tempered in those older men who maintain higher than average levels of testosterone. Additional studies, including work on human female pituitary function and responsiveness to physiologic doses of estrogen, are being initiated.

Further work on the mechanism of loss of adrenal hormone (glucocorticoid) responsiveness by rat adipocytes has focused on the biochemistry of the glucocorticoid receptor from these cells. A protein which has the characteristics of glucocorticoid receptors has been partially purified and characterized. The stability of the messenger RNA for this protein has also been studied and has been shown to have a half-life of approximately two hours in 12 month old rats. Similar studies in old animals are pending. The question of oxidation in cell membranes as a mechanism of aging has been further investigated, and a progressive oxidation of membrane bound sulfhydryl groups has been documented. Work on cardiac adrenergic β-receptors has shown no changes in membrane receptor number with age, nor in the increase in number of these receptors with administered thyroid hormone. New projects which have been initiated include investigation of mechanisms of corticosteroid mediated effects on rat cardiac muscle sensitivity to catecholamines, the effects of age and sex steroids on rate of skin healing, and the relationship of reduced lipolysis in response to fasting and to the increase in body fat which occurs with age.

The research efforts of the Cardiovascular Section fall into the general categories of human and animal research.

Research in humans involves non-invasive assessment of cardiac performance. Earlier echocardiographic work from this laboratory has shown that the left ventricular thickness and aortic root diameter increase as a function of age in normal man, whereas cavity size, ejection fraction and velocity of shortening are unchanged and mitral valve closure slope is reduced. More recent results demonstrate that when beta-adrenergic tone is eliminated, the left ventricular cavity dilates in response to phenylephrine more in the aged than in the adult population. The results of this work have appeared in two recent publications: (1) Yin, F. C. P., Raizes, G. S., Guarnieri, T., Spurgeon, H. A., Lakatta, E. G., Fortuin, N. J., and Weisfeldt, M. L.: Age-associated decrease in ventricular response to haemodynamic stress during beta-adrenergic blockade. Brit. Heart J. 40:1349-1355, 1978, and (2) Lakatta, E. G.: The cardiovascular system and aging. In Busse, E. D. (Ed.): Cerebral Manifestations of Episodic Cardiac Dysrhythmias. Princeton, Excerpta Medica, 1979, pp. 51-59.

In other studies, the prevalence of supraventricular and ventricular ectopy of all types, conduction disturbances, disorders of impulse formation and repolarization of the ECG is obtained over a 24-hour period via a 2-channel Holter monitor in men and women in the Baltimore Longitudinal Study. We have found that supraventricular and ventricular ectopy occur to a greater extent in a healthy active geriatric population than in persons of younger age as reported previously.

Other research projects in humans focus on the long-term therapeutic efficacy of pharmacologic agents. Despite the nearly universal employment of digitalis in the treatment of chronic congestive heart failure (CHF), there has been no objective documentation of its long-term efficacy. In a double blind crossover fashion, the effects of maintenance digoxin therapy in chronic stable CHF, utilizing clinical findings, chest radiography, echocardiography and systolic time intervals to assess baseline left ventricular function non-invasively and maximal exercise testing to determine aerobic work capacity is currently in progress. Results on the first 30 subjects, who are representative of a large segment of all those treated with cardiac glycosides, indicate that digoxin has no demonstrable long-term effect on either clinical status or indices of cardiac muscle and pump function. These results will be presented at the American Heart Association Scientific Sessions later this year.

Studies on various aspects of cardiac glycosides link part of our human studies to our research in animals. We have examined the role of aging on the: (1) direct effect of cardiac glycosides in isolated cardiac muscle from rats; (2) effect of cardiac glycosides on electrophysiology and pump performance in intact dogs; and (3) effect of cardiac glycosides and Na^+-K^+ ATPase inhibition in cardiac muscle from both rodent and canine species. The results indicate that in both the awake beagle and in cardiac muscle isolated from the rat, the inotropic efficacy of cardiac glycosides is markedly diminished in senescence. Furthermore, this diminution in effect cannot be attributed to an alteration in its ability to inhibit Na^+-K^+ ATPase, the purported glycoside receptor. In addition, the threshold for glycoside toxicity as manifest in ventricular tachycardia is *not* age related. The results of these studies have recently been published: (1) Gerstenblith, G., Spurgeon, H. A., Froehlich, J. P., Weisfeldt, M. L., and Lakatta, E. G.: Diminished inotropic responsiveness to ouabain in aged rat myocardium. *Circ. Res.* 44:517-523, 1979, and (2) Guarnieri, T., Spurgeon, H. A., Froehlich, J. P., Weisfeldt, M. L., and Lakatta, E. G.: Diminished inotropic response but unaltered toxicity to acetylstrophanthidin in the senescent beagle. *Circulation* (In Press).

The role of biochemical mediation of the contractile response in isolated cardiac muscle is addressed by collaborative efforts between the Cardiovascular and other laboratories at the Gerontology Research Center. Previous work from this laboratory has demonstrated that, when compared to the young adult rat myocardium, that from aged rats exhibits a diminished inotropic response to catecholamines. Our current results indicate that this difference cannot be ascribed to age changes in the beta-receptor or generation of cAMP or to activation of protein kinase but rather appears to result from a diminished extent of phosphorylation, and thus a diminished increase in Ca^{++} transport in the senescent versus the adult heart. Further testing on this hypothesis is currently underway in measurements of transsarcolemmal Ca^{++} influx as reflected in the transmembrane action potential in working cardiac muscle.

Other significant studies of the intersectional, inter-laboratory consortium have been in investigation of the hyperthyroid myocardium. We have demonstrated an increased contractile response to threshold concentrations of catecholamines accompanied by an increase in protein kinase stimulation in hyperthyroid versus euthyroid hearts. This provides a mechanism to explain in part the apparent hyperadrenergic state in hyperthyroidism and also provides a logical explanation why treatment of hyperthyroidism with beta-adrenergic blocking agents has been successful. These results have been submitted for publication.

In other studies in isolated cardiac muscle, we have sought to elucidate the basic mechanisms that govern the contraction process.

Starling's law of the heart, or increased muscle performance at longer lenths, has according to "classical theory" in heart muscle physiology been attributed to the change in sarcomere geometry that accompanies the change in muscle length. Our studies indicate that an additional mechanism, length-dependence of contractile activation, accounts, at least in part, for the length-dependence of force production. The data, which indicate that transsarcolemmal Ca^{++} influx is dependent on muscle length, provide a mechanism which, at least in part, explains the length-dependence of excitation-contraction coupling in the cardiac muscle. The results of this study are in press: Lakatta, E. G., and Spurgeon, H. A.: Force staircase kinetics in mammalian cardiac muscle: modulation by muscle length. J. Physiol. (Lond).

Several functional alterations in the contractile process of cardiac muscle have been observed to occur with advanced age. Although the extent to which these functional alterations seen with senescence are related to the concurrent hypertrophy is unknown, a relationship is suggested since prolonged contraction duration, diminished sarcoplasmic reticulum function, and increased stiffness have been observed in experimental models of hypertrophy. We therefore produced cardiac hypertrophy of an equivalent degree seen in the senescent heart in the adult rat by banding the aorta. The results showed that the same spectrum of changes in both isometric twitch and viscoelasticity coefficients as those seen with senescence were demonstrated in these muscles from hearts hypertrophied by banding. However, the magnitude of the changes in the mechanical properties were still significantly less than those observed in senescence. While the findings of this study do not prove a causal relationship between hypertrophy and the functional alterations with senescence, they are suggestive that at least a portion of the functional alterations are due to the underlying hypertrophy. A portion of these results have been published (Lakatta, E. G.: Alterations in the cardiovascular system that occur in advanced age. Fed. Proc. 38:163-167, 1979) and others have been submitted for publication.

In summary, the research of the Cardiovascular Section, while broad in scope, all stems from our interest in elucidating both fundamental properties of and age-related changes in cardiac muscle, both in the isolated state as well as in intact man and animals. Some of our studies serve to link several facets (physiological, biochemical, pharmacological) of investigation in muscle, and is achieved in some instances by collaboration with other units. The insights gained from the research summarized above have encouraged us to continue to focus our efforts in these directions for the next several years.

The Metabolism Section has introduced a meticulously-developed assay for glycosylated hemoglobin into the Longitudinal Study list of variables. This measurement theoretically is a better estimate of tolerance to glucose under the conditions of daily living in contrast to tolerance under laboratory conditions. Studies on the interactions of obesity with race, sex, age, and glucose intolerance were expanded. The Pima Indian population has the largest known prevalence of diabetes in the world. They are being intensively studied by a collaborative effort of renowned laboratories both in the U.S. and abroad. We are conducting studies on the sensitivity of tissues to insulin and plan to expand these studies to examine beta cell responsiveness to insulin-secretogogues.

A subset of the BLSA subjects who show a peculiar and unusual anomaly of markedly discrepant oral and intravenous glucose tolerance tests have been studied by the IV-oral hyperglycemic clamp technique. They show low beta cell secretory responses to hyperglycemia (IV glucose) but normalized responses to oral glucose. This "correction" evidently is secondary to an enhanced secretion of gastric inhibitory polypeptide after glucose ingestion. Whether this interacting group of subjects will show any unusual patterns of disease, aging, or mortality in the future remains to be seen.

The rat perfused pancreas preparation has been standardized on "scrub" animals and is now being used on our aging colony rats to look for beta cell sensitivity changes with age. A defect has indeed been found--the dose:response curve of the beta cell to glucose is shifted rightward in the old rats but the maximal response is unchanged.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00001-09 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Metabolic Studies of Aging in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
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SECTION
Metabolism Section

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.5	2.5	3.0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The decline in glucose tolerance with age has been amply confirmed. The definition of levels of performance which should be considered abnormal at different ages remains in doubt. The mechanism of the decline is also under study. The relation of obesity to glucose intolerance is known. The effect of changes in obesity with aging on glucose homeostasis is under study. The possibility of a racial (i.e., genetic) factor in a high prevalence population (Pima Indians) is being investigated. In addition, characterization of subjects by their hemoglobin Alc levels is being explored since this value gives an index of the integrated blood glucose concentration over a period of months.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Hemoglobin A1 (HbA1), which contains a sugar moiety covalently bound to the β-chain N-terminal, has been shown by many studies to be a reliable indicator of time-integrated average blood glucose concentrations over a period of months prior to the HbA1 determination. We are presently measuring HbA1 levels in participants in the Baltimore Longitudinal Study. These determinations will be of use in addressing two major questions regarding glucose metabolism and aging: (1) Does the age-associated impairment in average performance on glucose tolerance tests indicate that the elderly actually have higher average time-integrated average blood glucose concentrations? (2) Do differences in individual average time-integrated glucose concentrations within a population have significant predictive value with regard to various physiologic and pathologic changes with age (e.g., cardiovascular disease, renal disease, etc.)? The poor glucose tolerance of obesity is attributable mainly to tissue insensitivity to insulin. This insensitivity can be quantified by the glucose clamp technique. Diabetes develops at a remarkably high rate in Pima Indians. Severe obesity is also common among them. This study examines the relation between obesity and diabetes in Pimas who are not yet diabetic by usual clinical standards, and examines the possibility that they have a genetic susceptibility to diabetes such that abnormalities in insulin sensitivity can be demonstrated at a stage of obesity when other racial groups remain normal.

Methods Employed: We are assaying HbA1 by cation exchange chromatography, using a modification of the techniques of Gabbay et al (J. Clin. Endocrinol. Metab. 44:859-864, 1977). The assay is very susceptible to several sources of variability and we have made several changes from previous methods in order to minimize intra- and inter-assay variation. This is particularly important since our primary aim is to examine differences within a normal population, and we have found that the range of normal HbA1 values is quite narrow. Among the methodologic improvements are techniques to control temperature and pH during the assay. Variance is also introduced by conversion of the oxy to the cyanmet species after elution. A reagent for rapid conversion of the Hb to cyanmet has been developed. The long-term storage of stored control samples has been thoroughly explored and practical methods have been devised. Monitoring of the elution pattern during a chromatographic run has also reduced intra-assay variability. Other methodologic improvements have increased the convenience and safety of the assay. These involve the use of small columns and use of a new buffer which reduces the cyanide by 85%. Subjects are participants in the BLSA.

For the obesity studies, the euglycemic insulin infusion glucose clamp has been used. Insulin is infused at a rate to create a hyperinsulinemic state of approximately 100 μU/ml. Plasma insulin, glucagon, and C-peptide are measured by radioimmunoassay. Subjects are BLSA participants, residents of the Gila River Indian community, and volunteers in the behavioral study of obesity being conducted at the Johns Hopkins Hospital.

Major Findings: Clearly the value of the HbA1 determinations as a predictor of various physiologic and pathologic end-points must await long-term follow-up. Our present cross-sectional data, however, do permit some observations: (1) The range of HbA1 values as a percent of total Hb in subjects in the BLSA population analyzed to date, screened for diseases and drugs affecting glucose metabolism, and for hematologic conditions affecting HbA1 levels (e.g., recent donation of blood, various anemias, etc.) is quite narrow (mean $\pm$ SD = 7.00 $\pm$ 0.55). The mean is similar to that seen in other studies, but the standard deviation is considerably smaller. At this point, it is unclear whether this reflects population differences or the emphasis on meticulous methodology in our studies. (2) There is a small but statistically significant cross-sectional increase with age in HbA1 values in the population analyzed to date. A linear regression on our data yields $y = 5.83 + 0.0216\ (A)$, $r = 0.676$, $p < 0.001$, where $y = \text{HbA1/TotalHb} \times 100$ and A = age in years. This increase in HbA1 most likely does reflect an increase in average glucose levels with age, since previous studies indicate no change with age in red cell population kinetics in man. There is an increase of 21% in mean HbA1 levels from age 20 to 80. In percentage terms, this cross-sectional increase with age is a much smaller change than the decline with age in oral glucose tolerance test performance seen in the BLSA population. (3) We have further explored our earlier finding that HbA1 shows a rapid oxidation to Fe^{+++} form under certain conditions. It was hypothesized that this might reflect a greater susceptibility to metal-ion catalysis of oxidation. Studies on purified HbA1c indicate that copper-catalyzed oxidation of HbA1c to the methemoglobin form proceeds more rapidly than that of HbAo. (4) Since the HbA1c as a percentage of total hemoglobin increases over the lifespan of the red cell, we have been able to use erythrocyte HbA1c content as an indicator of cell age. Studies of osmotic fragility of human red cells of different ages indicate that the oldest segment of the red cell population is bimodal with regard to fragility, containing both the most and the least fragile cells. The structural basis for this finding is unclear at the moment. However, the observation may be of use in elucidating changes in membrane properties with increasing age in cells of various types.

In the obesity studies, 56 euglycemic clamps have been performed (12 Indians, 7 Blacks, and 37 Caucasians). The studies on the Indians were performed at the Indian Hospital in Phoenix. Since the variables under study include two age groups, three degrees of obesity, three races, two sexes, and three degrees of glucose intolerance, a larger number of studies will need to be made before firm conclusions can be drawn with respect to the interactions among these variables.

Significance to Biomedical Research and the Program of the Institute: The remarkable prevalence (50%) of abnormal glucose tolerance in the older population of the United States coupled with the increased morbidity and mortality of patients with true diabetes mellitus demands a delineation of the effects of aging on the pathophysiology of carbohydrate homeostasis. The tests of "abnormality" in performance levels at different ages will be certain end-points which will develop with the passage of time: development of overt diabetes, retinopathy, coronary heart disease, and peripheral atherosclerosis; decline in creatinine clearance; mortality rate; rates of "physiological aging" in other organ systems.

Proposed Course: Continued multivariate analyses of the relation of glucose and insulin variables to the end-points noted above in the BLSA participants. Increase the number of subjects studied in the obesity effort. Finally, we wish to examine the kinetics of the reaction between glucose and hemoglobin in more detail. The reaction is commonly termed "irreversible" but some data from *in vitro* studies indicate that, in fact, it may be slowly reversible. Some clinical findings support this idea as well: in several studies, control of markedly elevated glucose levels in diabetics has produced a fall in HbAlc values more rapid than one would expect if the reaction were irreversible. We plan to incubate purified HbAlc in the absence of free glucose for prolonged periods to further investigate this problem.

Publications:

DeFronzo, R.A., Tobin, J.D., Rowe, J.W., and Andres, R.: Glucose intolerance in uremia: Quantification of pancreatic beta cell sensitivity to glucose and tissue sensitivity to insulin. *J. Clin. Invest.* 62: 425-435, 1978.

McGuire, E.A., Tobin, J.D., Berman, M., and Andres, R.: Kinetics of native insulin in diabetic, obese and aged men. Diabetes 28: 110-120, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00002-17 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Gastrointestinal Hormone Mediation of Insulin Release

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.5	2.5	3.0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study is designed to investigate the role of the gastro-intestinal tract as an endocrine gland in the maintenance of glucose homeostasis. Among the identified gut hormones, gastric inhibitory polypeptide (GIP) appears to be the major insulin secretogogue. The glucose clamp technique has demonstrated the role of the endogenously-released hormone under in vivo conditions in normal man. GIP infusion studies have shown that the hormone has the same effects when exogenously administered.

Project Description:

Objectives: The studies are designed to examine the role of the gastrointestinal tract on insulin responses to ingestion of certain nutrients in man. Responses to the endogenously-released and exogenously-infused hormone are compared. The perfused rat pancreas is used as a model for studies of age changes in insulin secretion.

Methods Employed: Subjects are normal volunteers. The glucose clamp technique provides control of the blood glucose concentration. Glucose and amino acids are measured by automated glucose-oxidase and chromatographic techniques. Insulin and GIP are measured by radioimmunoassay. Single pass rat pancreas perfusion via the Grodsky method is used.

Major Findings: In the perfused rat pancreas studies, young (11-13 mo) and old (24-26 mo) rats from the Gerontology Research Center have been studied. Pancreases were perfused in separate experiments at glucose levels of 80, 150, 220, and 360 mg/dl. Insulin responses were computed for the early phase (0-10 min) and the later phase (10-50 min) as well as for total response. The dose:response curve of the beta cell to hyperglycemia shows a shift to the right in the old rats, i.e., a decreased sensitivity to glucose. The insulin response is significantly decreased at the 150 mg/dl level, is lower but not statistically significant at the 220 level but is virtually identical in young and old rat pancreases at the 360 level. The age defect is greater in the early than in the late phase.

In the human studies, the GIP infusion studies have been published. Another manuscript has been prepared on a study of a small but interesting group of BLSA subjects who have markedly disparate performance on the oral and intravenous glucose tolerance tests; they dispose of the oral glucose load much more efficiently than they do the intravenous glucose. These 8 subjects were compared to an age- and obesity-matched group of BLSA subjects who do not show this discrepancy. The pathophysiology of this laboratory finding was clarified by the IV-oral hyperglycemic clamp technique. The disparate group showed low basal insulin, diminished insulin response to hyperglycemia induced by IV glucose, but correction of the insulin defect by a large insulin response to oral glucose. This was evidently accomplished via the GIP mechanism. The disparate subjects had a marked elevation of their plasma GIP levels after glucose ingestion.

Significance to Biomedical Research and the Program of the Institute: The high prevalence of altered glucose tolerance in aging and obesity, as well as the high incidence of adult-onset diabetes mellitus, require further understanding of factors which contribute to this process. In addition, an understanding of similarities and differences in the pathophysiology of the various categories of glucose intolerance provides the hope for more and improved methods of treatment. Pathological states associated with alterations in gastro-intestinal hormones are only superficially understood currently, and further investigation of these hormone systems adds greatly to a new area of medical knowledge.

Proposed Course: We anticipate an emphasis on manuscript preparation in the coming year. In addition, as women begin to return in significant numbers for second and subsequent visits to the BLSA, we will be studying glucose-insulin homeostatic mechanisms via the clamp technique in them. This test has not been performed on first visit subjects.

Publications:

Elahi, D., Andersen, D.K., Brown, J.C., DeBas, H.T., Hershcopf, R.J., Raizes, G.S., Tobin, J.D., and Andres, R.: Pancreatic alpha- and beta-cell responses to GIP infusion in normal man during controlled glycemia. Am. J. Physiol. In Press.

DeFronzo, R.A., Tobin, J.D., and Andres, R.: The glucose clamp technique. A method for quantifying insulin secretion and resistance. Am. J. Physiol. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00011-07 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Hormones, hormone receptors, and aging I. Aging and hormone-sensitive adenylate cyclase.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	None	

COOPERATING UNITS (if any)

Department of Surgery, Baltimore City Hospitals

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NIA, GRC, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies deal with influences of age on the biochemistry of hormone-sensitive adenylate cyclase in a variety of tissues. The purpose of these studies is to explore the mechanisms of age-related alterations of hormone responsiveness and biological membranes, with special emphasis on the relationship between adenylate cyclase and hormone receptors.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project explores the mechanisms by which aging affects the hormone-sensitive adenylate cyclase system. A number of hormones exert their actions at the level of the cell surface by initiating a series of biochemical events which lead to the characteristic expression of the hormone's action. Such hormones include a number of small polypeptides (glucagon, thyrotropin, etc.), the catecholamines (norepinephrine, epinephrine, and dopamine), histamine, and serotonin. In each instance the hormone interacts with a specific chemical entity termed a "receptor". This interaction leads to the activation of an enzyme, adenylate cyclase (AC), which promotes the conversion of adenosine triphosphate (ATP) to adenosine-3'-5' monophosphate (cAMP). cAMP in turn activates any number of protein kinases, a process which leads to initiation or acceleration of many biochemical events.

The biochemistry of adenylate cyclase, a multi-component, membrane-bound enzyme, is rapidly being elucidated. Receptors are being quantitated and isolated, as are other components of the system. As a result we have in recent years undertaken a series of investigations that aim to a) identify those AC mediated hormonal responses that are age-dependent b) elucidate the biochemical mechanisms by which the changes occur. In this latter aspect, we are specifically attempting to quantitate the components of the activating system, i.e., receptors, AC, and the control of their interrelationships or "coupling". Since the hormone receptors and AC are integral parts of the cell's outer membrane, our work is, in a real sense, a probe of the effects of aging on cell membranes. Age-related changes of epinephrine, glucagon, and ACTH-sensitive adenylate cyclases in liver and fat have already been described from our laboratory.

Methods employed: Tissues used are from experimental animals (mainly the rat) and from man (surgical or necropsy specimens). Tissue homogenates, isolated cells, and cell membranes are employed, cells being isolated by collagenase digestion and membranes by density gradient centrifugation, etc. Adenylate cyclase is quantitated by a labeled substrate assay in which α-^{32}P ATP is converted to a α^{32}-cAMP and the latter quantitated after isolation on columns. Use of two marker isotopes (^{3}H-cAMP and ^{14}C-cAMP) enables precise correction of losses due to destruction during incubation and chromatography. Receptor quantitation utilizes labeled hormone antagonists. Protein factors are isolated by standard techniques of protein fractionation (gel and ion-exchange chromatography, etc.).

Major Findings: 1) Cytosol factors required for adenylate cyclase activity. We previously noted an increase of epinephrine-sensitive adenylate cyclase in rat liver during aging. During that work, loss of epinephrine and glucagon-sensitive adenylate cyclase activity was noted during preparation of membranes from crude homogenates. The mechanism of loss is partly due to the removal of GTP but also to removal of one or more proteins which interact with the membranes to stimulate hormone-sensitive cyclase. These proteins have been partially characterized and a technique for their quantitation developed. Our results to this point have now been published.

Further work on these factors has also continued, but efforts to isolate the materials have met serious difficulties. Although the crude material is quite stable, purification by ammonium sulfate precipitation and gel filtration results in marked loss of activity. The glucagon stimulating activity (GSA) seems to be distinct from that which activates epinephrine (ESA). On gel filtration (Sephadex G-100) GSA appears as two peaks, raising the possibility of monomer-dimer association or dissociation as the phenomenon responsible for the inactivation. Other chromatography experiments also point to serious losses during isolation. Efforts to prevent these losses have been unavailing to date and have prevented further isolation progress for the present. However, additional characterization has continued. Attempts to isolate the factors by affinity chromatography on Sepharose-GTP have given evidence for absorption, but the specifity of this phenomenon remains to be shown. Thus, the relationship of the factors to the GTP binding protein which is involved in AC regulation remains unclear.

The possibility that the factors found in "cytosol" are in fact derived from constituents of the cell membrane has been considered. Membranes, washed free of cytosol, have been further broken and extracted to yield significant amounts of activator proteins. Lactic dehydrogenase has been used as a marker for cytosol proteins. Preliminary results suggest that contamination of the washed membranes with proteins is minimal. All of this suggests that the "cytosol factors" are actually derived from membranes.

In addition to the possibility that the cytosol factors are actually derived from the membrane, we have performed several preliminary experiments to test the notion that membranes in general may contain an activator. Human red cells which contain little or no AC do contain a membrane activator of enzymes similar to AC, i.e., the ATPases. In preliminary experiments we now find that red cell membrane extracts do appear to activate hepatic AC. An intensive effort will soon further explore this possibility.

2) Concentration of β-Receptors in Rat Liver and Fat Cells. a) Liver. As reported last year, the concentration of β-adrenergic receptors in liver cells is much lower than that of other tissues. Although some workers have reported measurable quantities in livers from adult rats, most have used very young animals which have been adrenalectomized. We have now been able to obtain stereospecific binding with two antagonist ligands (^{125}I-hydroxybenzylpindolol and ^{3}H-dihydroalprenolol (DHA)). However, we can only obtain such binding in very young (30-day weanling) animals or young animals which have been adrenalectomized. β-binding quantitation in adult animals has been uniformly unsuccessful. We conclude that much published work in this area is incorrect. A paper dealing with these problems has been prepared.

b) Fat Cells. No difficulty was encountered in this area. Stereospecificity was established. Scatchard plots analyzed by computer revealed binding sites of much higher affinities than have been reported by others. It is our present belief that much of the earlier work on β-receptors of fat cells has dealt with non-specific binding. A manuscript dealing with this methodologic problem is in preparation.

3) Age-Related Alterations of Lipolysis in Rat Fat Cells. A number of studies have suggested that in the rat β-adrenergic receptor mediated lipolysis decreases with aging. The best of these studies has not yet been published in definitive form. Nonetheless, we undertook an examination of this issue in order to attempt to identify the mechanism of the decreased responsiveness.

β-adrenergic receptors were quantitated by DHA binding. Lipolysis dose-response curves (glycerol release/10^6 cells) were established with cells from animals of 2, 6, 12 and 24 mos. Lipolytic response was age-related and showed progressive increase in concentration needed for lipolysis between 2, 6, and 12 mos. By 24 mos. this trend reversed: the sensitivity increased and lipolysis occured at lower concentration. This change correlated with decreasing intracellular cAMP formation measured at the K_a (50% activation concentration) of epinephrine. However, β-receptors (f moles/10^6 cells) and their affinity were not significantly decreased with age. Similarly, AC was unchanged. At very high epinephrine concentrations cells from the old animals showed unexpected increase of cAMP. Since it is cAMP that mediates lipolysis and cAMP decreases even as sensitivity to epinephrine increases, an explanation for the age-related lipolysis must be found in events other than those which are demonstrable in the adenylate cyclase system. Further analyses of the data are in progress.

Significance to Biological Research and the Program of the Institute. Our ongoing studies are producing basic information upon which depends an understanding of age-related changes of hormone sensitivity. In addition, new insights are being obtained concerning the biochemistry of receptors and adenylate cyclase.

Proposed Course of the Project: Further characterization and purification of the "cytosol" adenylate cyclase activators will be attempted. An effort will be made to examine the role of diet in the age-related alteration of lipolytic response.

Publications:

Katz, M. S., Kelly, T. M., Piñeyro, M. A. and Gregerman, R. I.: Activation of epinephrine- and glucagon-sensivitive adenylate cyclases of rat liver by cytosol protein factors. Role in loss of enzyme activities during preparation of particulate fractions, quantitation and partial characterization. J. Cyclic Nucleotide Res. 5: 389-407, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00012-07 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Hormones, hormone receptors and aging. II. Aging and hormone responsiveness.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. S. Roth	Research Chemist, CPB, NIA
OTHER:	W. C. Chang	Visiting Fellow, (resigned 12/31/78), CPB, NIA
	H. Ito	Visiting Fellow, (EOD 5/1/79), CPB, NIA

COOPERATING UNITS (if any)

D. D. Schocken, Cardiology Fellow, Duke University, School of Medicine

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SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NIA, GRC, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is mainly involved in relating age, the various components mediating hormone actions (including receptors) and the biological responsiveness of hormone sensitive tissues.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project attempts to elucidate the mechanisms by which the actions of hormones are altered during aging.

Methods Employed: Whole animals, isolated tissues, and defined cell population in short term culture are used. In addition, white blood cells are obtained from Longitudinal Study participants in accordance with Human Research Committee guidelines. Hormone receptors, located either on the cell surface or intracellularly, are studied qualitatively and quantitatively by measuring specific binding of labeled steroids, catecholamines and other hormones to tissues, cells and subcellular fractions. Affinity chromatography is used to isolate hormone receptors for measurement of synthetic and degradative rates as well as purification for preparation of antisera and immunochemical titration. Hormonal control of various cellular metabolic processes such as nutrient transport and utilization are measured by standardized techniques. Macromolecular biosynthetic processes are also assessed.

Major Findings: 1) We have continued our use of the rat epididymal fat pad adipocyte as a model for post-mitotic cellular aging. Previously, we reported that decreased glucocorticoid responsiveness of these cells during aging was closely linked with reduced levels of glucocorticoid receptors and a reduced rate of synthesis of a protein having the characteristics of the glucocorticoid receptor. In the past year we have accumulated more data which suggest that this protein (or proteins) may indeed be the adipocyte glucocorticoid receptor. Purification is in excess of 2000 fold and may be considerably higher since putative receptor preparation protein concentrations are below the range detectable by standard assays, even when large amounts of purified material are concentrated. This protein has now been found to possess a molecular weight of about 50,000 at low ionic strength and migrates as a single bond on SDS-gel electrophoresis. These properties are comparable to those of glucocorticoid receptors purified from liver by others (other properties of the putative adipocyte receptor including binding affinity for dexamethasone, sedimentation coefficient etc. were reported last year to be comparable to those of various glucocorticoid receptors).

It has also been possible to determine the approximate half life of the messenger RNA for the putative adipocyte glucocorticoid receptor by using cordycepin, an antimetabolite which prevents polyadenylation and transport of newly formed mRNA out of the nucleus. Cordycepin has very little effect on intrinsic mRNA stability, a problem previously reported for actinomycin D. Using this method we have determined a half life of about 2 hours for the putative receptor message in adipocytes of mature, 12 month old Wistar rats.

We have attempted to determine whether the effects of age on adipocyte glucocorticoid receptors and response are intrinsic to the cells or due to some hormonal factor(s) present in the "milieu interieur" of aged rats. Adipose tissue has been cultured in explant systems as well as transplanted in vivo in millipore diffusion chambers and maintained for one week. Unfortunately, no experiments utilizing senescent tissue have yet been performed since even young tissue loses responsiveness to dexamethasone (assessed by hormonal inhibition of glucose metabolism) after 24-28 hours. This loss of responsiveness is unlike the loss characteristic of senescence in that explants and transplants show a 2-3 fold increase in basal glucose utilization. The tissue appears to be glucose starved and resistant to any attempts (such as by glucocorticoid treatment) to slow down glucose metabolism.

Last year we reported also that the membrane transport system for glucose of aged adipocytes becomes increasingly resistant to regulation by both stimulatory and inhibitory agents (insulin, vitamin K_5, H_2O_2 and glucocorticoids). Since the oxidation state of membrane protein sulfur groups has been implicated in control of glucose transport, and oxidation has been proposed as a possible aging mechanism, we have now measured both total and reducible sulfur groups on the adipocyte cell surface. Titration with radioactive mercury-dextran (J. Pitha and B. Hughes) reveals a progressive loss of reduced (-SH) sulfur groups with increasing age although total sulfur group concentration is essentially unchanged.

2) We have now completed our examination of cardiac β-adrenergic receptors and their control by thyroid hormones during aging. Binding of ^{3}H-dihydroalprenolol to these sites is sterespecific for (-) isomers of catecholamines, and saturable at very low ligand concentrations ($K_D \approx 5$ nM). No age changes in receptor concentration have been observed. The impaired ability of β-adrenergic agonists to stimulate cardiac contraction in aged rats (Lakatta et. al., 1975) appears therefore to be due to changes in factors distal to the receptor. Administration of thyroid hormones for 7 days in vivo causes an approximately 50% increase in cardiac β-adrenergic receptor concentration, but no change in K_D. Contractile responsiveness to calecholamines is also increased. The magnitude of this effect is identical in 9-12 and 22-26 month old Wistar rats. Thus, no age changes in cardiac β-adrenergic receptor levels or ability to regulate them by thyroid hormones are evident during aging of the rat.

Sensitivity to catecholamines in the rat heart also has been reported to be under possible glucocorticoid control. Preliminary experiments in collaboration with the Cardiovascular Section reveal that adrenalectomized young rats exhibit a markedly decreased sensitivity to Ca^{++} transport in various tissues including muscle. Since catecholamines cause cardiac muscle contraction via stimulation of Ca^{++} influx, possibly altered regulation of Ca^{++} transport by glucocorticoids during aging may play some role in functional changes in cardiac contractility.

3) We have begun preliminary examination of two complex physiological processes which are under hormonal control and have been suggested to change during aging. These are wound healing, and control of relative adiposity.

Several reports suggest that wound healing is potentiated by sex hormones. Our preliminary findings are in agreement in that estrogen replacement in 5 month old ovariectomized female rats appears to accelerate the healing time of 2 cm square full thickness skin lesions. Healing time is greatly increased in 22-24 month counterparts and estrogen replacement seems to be ineffective. Thus older rats appear to heal more slowly, possibly due to decreased estrogenic sensitivity and/or levels.

Data from our Longitudinal study suggests that with increasing age proportionally more of the ingested calories are deposited as fat and proportionally less as muscle. Relative obesity also increases with age (although partially due to muscle loss). In vitro studies by several groups suggest that older/larger adipocytes may be less sensitive to lipolytic hormones. To explore whether altered lipolytic sensitivity may play a role in the age related increase in obesity, we have fasted rats of different ages for 5-6 days and determined body weight, relative obesity, epididymal depot size, adipocyte size, catecholamine and insulin levels. Although the actual physiological mediator of lipolysis has not been conclusively identified, catecholamines are believed to play an important role, while insulin exerts anti lipolytic effects. During preliminary fasting experiments, we have observed no age differences in the levels of these hormones in peripheral blood (S. Tzankoff, D. Elahi). However, 12 and 24 month old rats lose relatively only about one-third as much of their total weight and fat content as do 2-3 month old animals. Thus altered sensitivity to these lipolytic modulators may be related to changes in fat composition and metabolism during growth and aging.

Significance to Biomedical Research and the Program of the Institute: Altered responsiveness to hormones is characteristic of a generalized functional decline during aging. Elucidation of those mechanisms by which hormone actions change with age is essential to any understanding of senescence. Accumulation of such basic information may allow prevention or reversal of some effects of aging by appropriate endocrinological, pharmacological or biochemical intervention.

Proposed Course of the Project: 1) The molecular mechanisms responsible for decreased glucocorticoid responsiveness, receptors, and putative receptor synthetic rate will be further examined in aged adipocytes. It will now be important to compare the half life of putative receptor messenter RNA in young and old cells to determine whether age changes occur at this level. Attempts to elucidate those factors which control glucocorticoid receptor synthesis, as well as attempts to raise antisera against these receptors, will continue. Specific antisera will then be used to determine whether nonfunctional, but still immunoreactive receptors can be detected in senescent cells.

Attempts will be made to determine why glucocorticoid responsiveness is lost in fat explants and transplants, and modifications of these systems to overcome the problem will be sought. If such modifications can be successfully made, explants of young and old rats will be cultured in young and old sera, and transplants into young and old hosts will be attempted. In this way it may be possible to determine whether adipocyte aging is "intrinsic" or dependent on the cellular environment.

Studies on the oxidation state of adipocyte membrane sulfur groups will be expanded to determine the effects of hormone treatment as a function of age. Since insulin may regulate glucose transport by altering the oxidation state of transport protein sulfur groups, it will be of interest to determine whether age related increases in oxidized groups play a role in reduced insulin sensitivity. Whether other factors which regulate glucose transport also act through this mechanism also needs to be determined, in light of the age and or size related loss of membrane sensitivity to such agents.

2) The possible role of altered glucocorticoid sensitivity in regulating the contractile response of aged myocardium will be explored further. Regulation of calcium transport and contractility by glucocorticoids will be examined in hearts of young and old rats both before and after catecholamine stimulation.

3) Studies on hormonal control of wound healing and adiposity will be continued in attempts to confirm our preliminary observations. We will attempt to identify the step at which estrogen seems to potentiates the wound healing process and further examine the possible causes of insensitivity in aged rats.

It will be of interest also to determine whether rats subject to controlled caloric intake and excercise levels exhibit the same age changes in control of relative adiposity as those subject to vigorous fasting conditions. In this regard, it may be useful to set up a cyclopropane chamber for measurement of total body fat without physical invasion or harm to the animals. The effects of age and size also need to be separated in attempting to interpret such experiments.

Publications:

Roth, G. S., Chang, W-C. and Gesell, M. S.: Changes in Hormone Receptors During Aging: Role in Altered Hormonal Responsiveness. In Vassileva-Popova, J. (Ed.): Biophysical and Biochemical Information Transfer in Recognition. 1978, p. 123-138, Plenum, New York.

Roth, G. S. and Tata, J. R.: Receptors, Recognition and Aging. In Vassileva-Popova, J. (Ed.): Biophysical and Biochemical Information Transfer in Recognition. 1978, p. 835-851, Plenum, New York.

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Joseph, J. A., Berger, R. E., Engel, B. T. and Roth, G. S.: Age Related Changes in the Nigrostriatum: A Behavioral and Biochemical Analysis. Journal of Gerontology 33: 643-649, 1979.

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Roth, G. S.: Hormone Receptor Changes During Adulthood and Senescence: Significance for Aging Research. Federation Proceedings 38: 1910-1914, 1979.

Schocken, D. D. and Roth, G. S.: Age-associated Alterations in Adrenoreceptors. In Syabadi, E., Bradshaw, C. M. and Bevan, P. (Eds.): Recent Advances in the Pharmacology of Adrenoreceptors. 1978, p. 227-232, Elsevier, North Holland.

Schocken, D. D. and Roth, G. S.: Age-associated Loss of Beta Adrenergic Receptors. In Cristofalo, V. J., Roberts, J. and Adelman, R. C. (Eds.) Pharmacological Intervention of the Aging Process, Plenum, New York, in press.

Roth, G. S. and Livingston, J. N.: Dissociation of Glucocorticoid Inhibition of Glucose Transport and Metabolism by Increase in Adipocyte Age and/or Size. Endocrinology 104: 423-428, 1979.

Chang, W-C. and Roth, G. S.: Changes in the Mechanisms of Steroid Action During Aging. J. Steroid Biochem., in press.

Roth, G. S.: Aging and Hormonal Receptors. The Naito Intl. Symposium on Aging, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00013-05 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Hormones, hormone receptors, and aging. III. Aging and the human male reproductive system.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S. M. Harman, Senior Investigator, CPB, NIA

OTHER: P. D. Tsitouras, Visiting Associate, CPB, NIA
C. E. Martin, Senior Investigator, CPB, NIA

COOPERATING UNITS (if any)
NICHD, RRB

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Endocrinology Section and Human Performance Section

INSTITUTE AND LOCATION
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TOTAL MANYEARS: 2.1	PROFESSIONAL: 1.1	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of tests of reproductive endocrine function are being carried out on male volunteers in the Baltimore Longitudinal Study on Aging. Cross sectional and longitudinal data have been obtained which deal with testosterone and estrogen levels in plasma. Pituitary function is being assessed with LHRH stimulation of plasma pituitary gonadotrophins. Level of sexual activity is being related to the hormone assays.

Project Description:

Objectives:

A. Background - Steroid hormones secreted by the gonad play an important role in regulating body economy throughout the lifespan. In the male, testosterone, the major testicular steroid hormone, not only maintains secondary sex characteristics and sexual function, but is responsible for positive nitrogen balance and maintenance of increased muscle mass, for skeletal integrity, and possibly for such diverse functions as rate of healing and general level of aggressiveness. Steroid secretion by the testis is maintained by the pituitary hormone, LH, which is itself regulated by hypothalamic secretion of another hormone LHRH. This intricate control mechanism is in turn inhibited by rising plasma levels of testosterone, forming a complete system with its own feedback device to assure constancy of function. A second pituitary hormone, FSH, also under hypothalamic regulation by LHRH, controls testicular production of germ cells.

B. Current knowledge - Alteration of function of the male reproductive system with advancing age has been reported by a number of investigators. Notable changes described include a decrease in the circulating level of testosterone after age 60, an increase in plasma protein binding of testosterone, which further lowers the level of "free" (and thus, presumably, bioavailable) testosterone, and an increase in circulating levels of female hormones (estrone and estradiol). At the same time the plasma FSH and LH levels have been found to increase, suggesting a primary failure of the testis, which releases the pituitary from feedback inhibition control. Some evidence also exists for a reduction in pituitary function with age in that response of the pituitary to exogenous LHRH appears to be somewhat reduced. Since study populations have not been well-defined in terms of variables which may affect hormone secretion such as nutrition and obesity, alcohol and tobacco consumption, and health and general level of activity, it is difficult to interpret the significance of currently available data to aging, per se. Furthermore, none of the available studies has a longitudinal design, which makes their interpretation subject to all of the difficulties which characterize cross-sectional aging studies. None of the available studies have attempted to correlate changes in sex hormone regulation with such variables as sexual behavior, prostate disease, cardiovascular disease, or subtle measures of psychological function.

C. Present study - Using a well-characterized group of men from the Baltimore Longitudinal Study on Aging, the effects of age on testicular function and pituitary-gonadal regulation independent of intercurrent illness, excess alcohol consumption, obesity, etc. is being defined. In addition, an attempt will be made to correlate altered function with the above variables and with changes in libido, coronary disease, and prostate disease.

Methods employed: (1) Plasma gonadotrophins are being assayed using a double antibody radioimmunoassay. (2) Plasma testosterone and dihydrotestosterone are measured with a very precise recently developed radioimmunoassay. (3) Plasma estrone and estradiol are assayed using a charcoal radioimmunoassay method. (4) Semen analysis has been performed by standard techniques of counting and staining. (5) The fraction of free testosterone in plasma is being estimated by an ion-exchange column method developed in our laboratory. (6) Blood samples are obtained from a healthy, non-obese subgroup of the BLS population before and after intravenous injection of 100 μg of LHRH to test for pituitary gonadotrophin reserve and then after intramuscular injection of human chorionic gonadotrophin to test for testis secretory reserve. (7) Freeze-dried plasma samples from the BLS subjects, taken in previous years, have been used to survey relationships between testosterone level and sexual function in collaboration with Dr. C. Martin. (8) Computerized techniques for regression and multiple regression analysis are used to evaluate data for associations of altered sex hormone secretion with other variables. (9) Subjects will be restudied at 4 year intervals to provide longitudinal data. Four cycles of study are anticipated.

Major Findings: 76 subjects over the age range 25 to 89 have participated to date. There are a minimum of 10 subjects in each decade. Due to a shortage of young BLS participants, it was necessary to recruit a small number of paid volunteers (age 2529) of comparable social and educational background.

Data show a significant increase in the basal FSH and LH levels with age, but, unexpectedly, no apparent change in total resting testosterone, dyhydrotestosterone, or estrogens. This combination of findings suggests that our subjects do experience some failure of testicular secretory mechanisms which is compensated for by the increased output of gonadotrophins. A likely explanation for differences between our findings and those of others may lie in non-comparability of populations investigated. Although the percent of testosterone bound to plasma binding globulin increases slightly but significantly with age, this is compensated for by a small rise in the total testosterone so that there is no net change in the free (i.e., bioavailable) testosterone index with age in our study. Analysis of testis secretory response to hCG suggests a diminished testicular reserve with age.

Pituitary function testing with LHRH shows no decrement in the absolute response of the pituitary for LH or for FSH in terms of peak or integrated (area under the secretion curve vs. time) response above baseline but a decreased percent response (response/basal) occurs. When a subgroup of young and old subjects matched for basal levels of LH are tested for absolute (peak or integrated) response differences, a difference between young and old groups is apparent, suggesting that there is a real alteration in pituitary gonadotrophic function with age. Because of the ambiguity of the above data further research is being conducted in collaboration with Drs. R. Sherins and D. L. Loriaux of the NICHD, RRB on the relationship of basal LH to LH response criteria after LHRH. Analysis of data obtained to date defines a direct relationship between basal LH (x) and LH response (y) which can be described by an equation of the form $\ln y = A + B (\ln x)$ in which both A (intercept) and B (slope) are positive numbers significantly different from zero. This relationship produces an obligatory decrease in percent response as basal rises which may be responsible, in part, for the diminished percent response observed in our aged men. Application of this new powerful technique to response data obtained from our aging study should further clarify its meaning.

Additional fundings include a small but significant age-related decrease in testicular size. Sperm analyses suggest that in our population the absolute number of sperm ejaculated does not decrease, but there is a trend toward increasing numbers of immature spermatozoa appearing in ejaculates with age.

Analysis of sexual activity information for our 76 subjects shows a weak but statistically significant correlation between sexual activity and free testosterone index in the oldest men (70-89) but not in the younger men. Measurement of testosterone in a set of 180 lyophilized samples, obtained from a separate group of 180 BLS men, ages 60-80, confirms this correlation and also the absence of a decrease in testosterone with age. In addition an inverse correlation between percent body fat and testosterone (but not sexual activity) was found. No correlation between testosterone and coronary artery disease, muscle mass, or reported prostate size was seen.

Measurement of the content in plasma samples (from the 76 volunteer cohort) of the α and β gonadotropin subunits has been completed and the data awaits analysis.

Significance to Biomedical Research and the Program of the Institute: Correla--tion of changes in sex hormones with behavioral, physiological or pathological status with age may shed light on mechanisms of various changes which have been observed in the aging male, including decreased libido and impotence, prostatic hyperplasia, and increased incidence of coronary disease (compared with the female). Normative data on levels of hormone secretion may help differentiate men with underlying disease processes from those who are simply aging.

Proposed Course: The second cycle of the above study was begun in summer 1979. Repeat testing will be done on all available BLS men from the first cycle. In this cycle serum prolactin measurements will be added to the previous protocol. New studies are contemplated in two additional areas. The first study will address the effects of stress of coming to GRC and of time of day on variation of testosterone level in blood in men of different ages. This study will use round the clock sampling to define diurnal variation and sampling of some men in their home or office environment prior to their arrival at GRC, in order to better define the meaningfulness of testosterone samples obtaine at GRC. Data obtained will be invaluable in interpretting previous results. The second area of study will involve measurement of metabolic rates of glycoprotein hormones, at first LH, and later possibly other hormones in men of various ages. These studies may help to clarify the meaning of the gonadotropin data previously obtained.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00023-04 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Hormones, hormone receptors, and Aging, IV. Aging and Leydig cell function.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S. M. Harman, Senior Investigator, CPB, NIA

OTHER: P. D. Tsitouras, Visiting Associate, CPB, NIA

COOPERATING UNITS (if any)
None

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The function of Leydig cells isolated from rat testis is studied in vitro in comparisons of old and young rats. Testosterone production, cell number of gonadotrophin receptors, cAMP production are assayed in order to localize the age-related alterations of Leydig cell function.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The Leydig cells of the testis secrete the major male hormone, testosterone, which is essential to normal male development and function, and which also has important actions at sites as diverse as bone, muscle, skin, and central nervous system. A number of studies have shown reduced function of Leydig cells both in animals and in man. Leydig cell function is normally under the control of the pituitary hormone, LH, which interacts with membrane receptors to activate adenylate cyclase. The resultant increase in cAMP leads to catalytic phosphorylation and activation of a system of protein kinases, which in turn alter the cell's internal metabolism and lead to hormone production and secretion. The goal of the present study is to define and investigate the nature of the defect appearing in an animal Leydig cell system with advancing age.

Methods Employed: Matched pairs of young (4-9 months) and old (22-26 months) rats from the GRC Wistar colony are killed by cervical dislocation and the testis removed and partially digested with collagenase. Tubular and interstitial elements are then separated by filtration, and, for cAMP experiments, Leydig cells are further purified by density gradient centrifugation. The number of viable Leydig cells in each preparation is estimated using a histochemical (3-ß-hydroxy-dehydrogenase) strain and the trypan blue exclusion technique. Short term incubations are then carried out with varying doses of human chorionic gonadotrophin (hCG, an LH-like hormone) to determine cell production of testosterone, cAMP, or cell membrane binding capacity and affinity for radioactively is analyzed by radioimmunoassay using Florisil for separation of bound and free hormone. cAMP present in washed cells (total) and bound to intracellular protein (presumably protein phosphokinase) are estimated by standard techniques of radioimmunoassay. Membrane receptor capacity is estimated from Scatchard plots obtained from incubations of Leydig cell membranes with radioactive (I^{125}-labelled) hCG.

Major Findings: Initial in vivo studies demonstrated that old male rats had only approximately 50% of the serum testosterone levels of younger rats. One hour of intravenous stimulation with hGC restored old rats' levels of serum testosterone, producing responses indistinguishable from those seen in young animals. Despite this restoration in vivo, old Leydig cells examined in vitro continued to demonstrate defective testosterone secretion after up to 180 minutes of hCG stimulation and regardless of dose, with approximately at 50% reduction in secretory capacity. Membrane hCG receptors showed a 30% reduction in old rats but intracellular cAMP and protein bound cAMP were comparable in young and old cells suggesting that the secretory defect is distal to the step at which cAMP activates protein phosphokinase, possibly involving enzyme activation or synthesis, substrate utilization, or intermediary metabolism (e.g., availability of reduced nucleotides, etc.). Presently ongoing work has indicated that pretreatment with hCG in vivo, followed by Leydig cell incubations produces percent decreases in membrane receptor capacity which are slightly, but significantly, greater in old than in young rats. This probably reflects reduced capacity of older cells to synthesize new receptors to replace those internalized and destroyed after hCG binding. Leydig cell responsivity to hCG is considerably reduced after hCG pretreatement in both old and young cells, but basal testosterone secretory rates are much higher than before treatment and appear comparable between young and old rats (the latter data are not complete).

Significance to Biomedical Research and the Program of the Institute: If the nature of aging is to be understood, the precise biochemical defects in the function of differentiated cells of aging animals as well as the defects hindering the replacement of such cells from populations of less differentiated cells must be investigated. Since the characteristic response pattern and details of many of the intermediate steps in the metabolism of Leydig cells is known, and since an aging defect in the function of these cells can be demonstrated and may have broad physiologic significance, this model system seems to be particularly suited for the study of aging processes.

Proposed Course: Work will continue in an effort to elucidate the nature of the "repair" process occurring in vivo, and to demonstrate the site or sites of the aging defect demonstrated in vitro in isolated Leydig cells. Young and old cells' performance will be compared in the presence and absence of various steroid precursors, cofactors, and energy sources. Rate limiting enzyme activities will be assayed, and studies of numbers, distributions, and structure of Leydig cells in situ before and after treatment using light microscopic and electron microscopic techniques will be undertaken.

Publications:

Tsitouras, P. D., Kowatch, M. A. and Harman, S. M.: Age related alterations of isolaxed rat Leydig cell function: Gonadotropin receptors, cAMP response, and testosterone secretion. Endocrinology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00014-09 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Biochemistry of renin and renin substrate.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: G. Pourmotabbed, Visiting Associate (resigned, 9-30-79), CPB, NIA
R. I. Gregerman, Chief, Endocrinology Section, CPB, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NIA, GRC, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project explores the biochemistry of renin and renin-substrate, proteins involved in hypertension. The substrate is being purified and labeled in order to develop a new assay for renin. Other studies include chemical modification of the substrate, isolation of renin inhibitors.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The enzyme renin, produced and secreted from specialized cells of the renal glomerulus, appears to be involved in several varieties of hypertension. Secretion of the enzyme is under control of a variety of physiologic and pathophysiologic factors, e.g., salt restriction, volume depletion, sympathetic nervous system control. In the circulation the enzyme acts on a plasma glycoprotein to release the decapeptide, angiotensin I, which is then activated by C-terminal cleavage to a smaller octapeptide, angiotensin II, in the lung and other tissues. Angiotensin II in turn influences the secretion of aldosterone, principal mineralocorticoid hormone of the adrenal, and has other direct effects on the cardiovascular and central nervous systems. In certain pathologic states renin has a direct role in the pathogenesis of hypertension, as it does in renovascular stenosis. Recent work suggests that renin may be more directly involved in common or "essential" hypertension. Preliminary reports indicate that an inhibitor of angiotensin II formation results in amelioration of hypertension in many patients. Finally, the secretion of renin (and aldosterone) is markedly influenced by aging in man, while hypertension is an age-dependent disease.

Progress in these areas has depended on advances in our knowledge of the basic biochemistry of renin and its substrate. Our own laboratory has been involved in the development of new techniques for measurement of the enzyme and its peptide products, in the biochemistry of the enzyme and its substrate, and the relevance of the renin-angiotensin-aldosterone system to normal and pathologic aging.

Methods Employed: Renin has been assayed by our previously published polymeric substrate assay and by immunoassays of angiotensin I. Renin substrate has been purified from porcine and human plasma by column chromatography on DEAE cellulase, Con-A Sepharose and Sephadex G-100. Other techniques are standard biochemical methods (amino acid analysis, high voltage electrophoresis, etc.).

Major Findings: I. Labeled Renin Substrate. Purified renin substrate prepared in our laboratory was labeled with ^{125}-Bolton-Hunter reagent. Much effort had been expended in preparing this material. Unfortunately, the labeled substrate was not cleaved by renin. This unexpected result is probably due to the occurence of ε-lysine NH_2 residues near the cleavage site. The groups, like the N-terminal NH_2, were probably labeled creating an unfavorable steric effect. Further efforts to produce a labeled substrate assay using such an approach have now been abandoned.

II. Kinetics of Renin Inhibition: Much of the previous kinetic analysis of inhibition used renin preparations contaminated with non-specific proteases. Since we have described a method for preparation of pseudorenin-free renin, we have attempted to use this material in a reexploration of this problem in order to resolve a number of discrepancies and criticisms which could be directed toward the earlier work. Sufficient quantities of the purified enzyme have now been prepared; kinetic analyses are now underway.

Significance to Bio-medical Research and the Program of the Institute. Ongoing work should clarify certain aspects of the inhibition of renin by inhibitors. These agents in turn may help clarify the nature of the hypertensive states and their biochemical bases.

Proposed Course of the Project: Work in this area will be terminated with the kinetic analyses. Studies will be resumed when new personnel can be recruited.

Publications: Chou, H. J., Rehfield, P. L., and Gregerman, R. I.: Linkage of the N-terminal peptide portion of renin substrate to the remainder of the protein. Evidence for an alkali labile Tyr-Ser and against an ester bond. Biochimica et Biophysica Acta 537: 366-373, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00015-21 CPB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Baltimore Longitudinal Study of Human Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Andres	Chief, Clinical Physiology Branch	CPB NIA
	A. H. Norris	Chief, Human Performance Section	CPB NIA
	N. W. Shock	Scientist Emeritus	NIA

OTHER: Other workers who are associated with the Longitudinal Study describe their involvement in their individual reports.

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Human Performance Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9.60	2.45	7.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Baltimore Longitudinal Study serves as a resource for scientists working in the field of Gerontology. It provides a well-described group of men and women between 20 and 96 years of age for studies of the mechanisms of human aging. Projects in physiology, biochemistry, psychology, nutrition, pharmacology, endocrinology, sociology, and genetics, have been carried out or are in progress.

PHS-6040
(Rev. 10-76)

Objectives: The Baltimore Longitudinal Study provides a well described group of subjects as a resource in support of a wide variety of scientific investigations in gerontology and other disciplines. While long-term planning is encouraged, important studies of shorter duration have also been undertaken. The long-term general goals of the project are to: (1) secure replicate measures of physiological, pathological, biochemical and psychological variables on longitudinal study participants at specified intervals; (2) summarize and compare the results of testing in relation to age according to cross-sectional and longitudinal formats; (3) identify characteristics of individual participants which may be related to changes of function over time and to age at death; and, (4) determine whether the data obtained support one or another theory of the mechanisms responsible for age-related functional decrements.

Methods Employed: The Sample: Study participants are male and female volunteers recruited by other participants in the program. Recruits agree to return to GRC in Baltimore for 2-1/2 days of testing every 12 months (age 70 and over), 18 months (age 60-69) or 24 months (under age 60) for an indeterminate period. At entry into the program, 86% of subjects reported at least some college, 87% were identified with professional, technical or managerial occupations, 90% were presently married, 83% described themselves as financially comfortable or better, and of the group who returned for the fifth visit, 90% rated their health as good or excellent on both first and fifth visits.

Data Management: Medical records and test results are maintained in written form in the laboratory and transferred to a data retrieval and analysis system by keypunching on tabulation cards or by recording the test results directly on punched paper tape or magnetic tape. Data are maintained and used in ways which protect the privacy of participants. Sensitive material is specially encoded. Individual scientists review, evaluate and summarize the data for scientific reporting.

Major Findings: By June 30, 1979 a total of 1108 men and 162 women have participated in the testing program on one or more visits to GRC. Since the inception of the study 200 men have died and another 265 have withdrawn from the program, leaving an active sample of 643 men. As of this date, 820 men have completed three or more visits for testing, 642 visited five times or more, 233 ten times or more, 111 twelve times or more, and 31 fifteen times or more. In all, these subjects account for a grand total of 6406 participant visits.

The first female subject was tested on January 4, 1978. Of the 162 women tested thus far, two have died and one has withdrawn from the program, leaving an active sample of 159. Seven women have had two visits for testing.

The intake of women into the BLSA is slightly higher than the rate of intake of men into the study at a similar stage in 1958-60 (108 women per year compared with 87 men per year). The induction of men into the study has reduced to 4 new men during the past year.

The active sample of men has not changed during the past year. It has been modified, however, by the activities of the Illness and Disability Study which has 1) identified participants not known to have died and 2) recruited dropouts to return to the study. This balance of results has occurred during the follow-up of participants over age 65. As the focus of the study moves to younger participants, there may be a net gain to the active group because fewer deaths will be found to balance the returnees.

All those subjects who failed to return to the BLSA as of July 1, 1979 and who were 60 years of age and over were contacted. Our priorities at first contact with the subjects were to seek: 1) Return to the BLSA for one visit, 2) Return to the BLSA for one day, 3) Home visit by the interviewer, 4) To send mailed questionnaire, and 5) Telephone interview only.

Ninety subjects were contacted. Eighty subjects were found to be alive (88.9%) and 10 subjects dead (11.1%). The age distribution of living subjects (n=80) as of July 1, 1979, is given in Table 1.

Table 1. Distribution of Subjects by Age

Age Group	No. of Ss.	% of Ss.
60-64	18	22.5
65-69	19	23.7
70-74	9	11.2
75-79	10	12.5
80-84	14	17.5
85-89	7	8.7
90+	3	3.7
Total	80	

Table 1 shows that a greater proportion of the subjects (46.2%) were in the 60-69 age group, while only 29.9% of the subjects were 80 years of age and over. The greatest number of deaths were found in the highest age groups.

The overall effect of the Illness and disability study on the net growth of the BLSA is shown in Table 2.

Table 2. Distribution of Subjects by Type of Response

Type of Response	No. of Ss.	%
1. Returned & will return	31	38.8
2. Mailed questionnaire	35	43.8
3. Home visit	11	13.8
4. Others	3	3.7
Total	80	

As shown in Table 2, the Illness and Disability Study has caused a net increase of 31 subjects in the 60 and over age group so far. This net increase for visits could have been more if transportation expenses were provided. Out of 35 subjects who agreed to respond to questionnaires, 19 stated that they would like to return, but they could not affort a trip to Baltimore. Of the remaining 16 subjects, 6 subjects have indicated that they would visit the BLSA for one or more tests later this year or next year.

Significance to Bio-Medical Research and the Program of the Institute: A major goal of the longitudinal program is a deeper understanding of age-related changes in the different organ systems, and their interrelationships. The relation of functional changes in an individual to age at death, age of onset of a disease, and other end points is important for understanding aging in humans and the impact of aging on society. The intensive study of multiple variables will also provide tests of risk-factor theories for specific age-related diseases.

Proposed Course: Data collection and analyses will be continued. Continued emphasis on automation of tests, data entry, and analyses should provide improved accuracy and efficiency. A major summary of all aspects of this program is in progress.

Publications:

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00016-24 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Age Changes in Human Performance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. H. Norris	Chief, Human Performance Section	CPB NIA
	S. P. Tzankoff	Sr. Staff Fellow	CPB NIA
OTHER:	N. W. Shock	Scientist Emeritus	NIA

COOPERATING UNITS (if any)
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S.M. Garn, Ctr. for Human Growth & Development, U. of Michigan, Ann Arbor,
G.A. Borkan, Boston Normative Aging Study, V.A. Outpatient Clinic, Boston

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.15	.85	3.30

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the mechanisms and the limitations of a variety of physical activities in old and young individuals. Muscular activity ranges from brisk walking on an inclined treadmill to tapping between targets of various widths drawn on paper and separated by various distances. Exercise responses are measured for blood pressure, heart rate, pulmonary ventilation, carbon dioxide elimination, and oxygen uptake. Responses of metabolites, such as lactic acid and hormones, such as catecholamines and growth hormone are measured. The oxygen cost of exercise is measured and compared to the total amount of physical work performed to estimate the mechanical efficiency of the subjects' neuromuscular and psychomotor control systems. Responses of the pulmonary system are interpreted in terms of standard spirometry and dead space (residual volume) measurements as well as studies of respiratory control. Limitation on performance imposed by cerebrovascular, cardiovascular, and pulmonary disease is assessed. Reflex time, reaction time and speed and accuracy of movement are measured and compared with exercise responses.

Project Description:

Objectives: This project is designed to study the effects of aging on the physiological responses to and recovery from exercise--to describe age changes and to elucidate the mechanisms of these effects of aging. It is designed to identify underlying factors in the limitation of work performance and reduced mechanical efficiency in older people. For this purpose, detailed evaluation of pulmonary function and pulmonary response to stressful agents are carried out. Other factors such as the metabolic cost of limb movement and psychomotor control of limb movement are being studied.

An additional goal is to identify and explain the role of disease-altered physiological function in age-related limitation of work performance. Cerebrovascular, cardiovascular and pulmonary disease and functional measures such as blood pressure, reflex time, and reaction time will be considered.

Methods Employed: Measured amounts of physical work are administered to subjects of varying ages by means of a calibrated arm ergometer and quantitative mechanical analysis of limb movement. A treadmill is used to induce higher levels of work. Measurements of oxygen uptake, CO_2 elimination, pulmonary ventilation volume, heart rate, blood pressure, and electrocardiogram are made before, during and after standardized amounts of exercise. The functional capacities of the pulmonary system are evaluated. Alterations in respiratory function as a result of the stimulation of low oxygen and high oxygen and carbon dioxide in the inspired air are evaluated by pressure changes induced by occlusion of airflow ($P_{0.1}$).

Subjects of the BLS continue to be evaluated for participating in the multi-purpose maximal treadmill exercise tests. Subjects are instructed to walk on the motor-driven treadmill at a constant speed of 5.6 km/hr. They start on the level and the grade is elevated by 3% increments every two minutes until either exhaustion or, at the discretion of the attending physician, the test is terminated. Before the walk, as well as during the exercise and recovery from it, electrocardiographic tracings are displayed and monitored on a CRT, recorded on magnetic tape, and periodic samples reproduced on strip-chart paper. In addition while walking, subjects breathe through a mouthpiece-valve arrangement which allows for the inspiration of room air and expiration into spirometers for measurement of pulmonary ventilation, and after gas analyses, for the calculation of oxygen consumption, carbon dioxide production, and the respiratory exchange ratio for each level of exercise.

In the recovery phase venous blood samples are obtained at 3, 5, and 7 min. for the determination of lactic acid concentration, a by-product of anaerobic metabolism. Each subject who progresses through the test until exhaustion is asked to identify his limiting symptom, e.g., muscle pain, shortness of breath, general fatigue, and this response recorded. In addition, the investigator makes a subjective evaluation regarding whether or not the performance represented a maximal effort. Exercise and recovery electrocardiographic tracings are evaluated for signs of ischemic coronary heart disease (ICHD) according to the World Health Organization standards.

Major Findings: Details of a study of metabolic responses of primates to exercise and behavioral heart rate conditioning which involves collaboration with LBS is reported in Z01 AG 00063-12 LBS.

Details of a study of metabolic and adrenergic response to exercise with and without behavioral heart rate conditioning which inolves collaboration with LBS is reported in Z01 AG 00067-12 LBS.

BIOLOGICAL AGE PROFILES RELATED TO APPEARANCE OF AGE AND SURVIVAL. In most of the previous studies of biological age in adulthood the data for a wide variety of age-related variables have been reduced to a single score by multiple regression analysis. Few studies have utilized their results in further investigation, and none have attempted to validate their technique. The present approach was based on the view that there may be many different biological ages in the same individual, requiring analysis of a profile of biological age scores. The relative importance of certain performance variables was discussed in last year's report.

This investigation was based on cross-sectional data for 1086 adult male participants in the Longitudinal Study of the Gerontology Research Center in Baltimore, Maryland. Twenty-four variables were selected as representing a variety of aspects of physical aging. The raw data were transformed into biological age scores which reveal the extent to which a man had traits more similar to men chronologically older or younger than himself for each variable. These results were expressed in standard (z-score) units enabling all chronological ages and all variables to be analyzed together. The technique of pattern profile analysis was adopted to graph these data in a readily interpretable manner.

In order to evaluate this technique the mean biological age profiles of two sets of subpopulations within the data were compared. The first parameter divided the sample into men who were estimated by physicians as looking older than their age or younger than their age. It was hypothesized that men who looked older would have greater biological age on the 24 profile parameters as well. This analysis showed that the hypothesis was valid for 19 of 24 variables with 6 significant differences in the expected direction. The agreement between these two essentially independent techniques of estimating biological age supports the validity of the approach. The results also suggest that the observation that some people look older than their age has a foundation in a wide variety of physiological measures.

A second approach to subdividing the sample compared men who died since the study began (166 individuals) with men who have survived. The hypothesis that men who were sooner to die would have had greater biological age was supported by the results. Nineteen of the profile variables showed the hypothesized relationship and nine of these were significant differences. These results also support the view that many of the biological age profile parameters are aspects of aging which are related to survival. Further analyses using this technique can examine whether there are differences in biological age between subpopulations based on such things as health, lifestyle, and nutrition.

Significance to Bio-Medical Research and the Program of the Institute: The decline of the ability of some older people to perform their day-to-day activities and to engage in pursuits which contribute to the economic and social strength of our society represents a national loss. Identification of the physiological, medical and social correlates of high levels of physical strength and psycho-motor performance in middle and old age, as well as declines in these abilities, should lead to techniques designed to reduce the rate of decline in performance capacities with age.

Proposed Course: Measurements of muscle strength and maximum power generating ability during arm exercise will be continued. Cardiovascular, ventilatory and metabolic responses to standardized arm ergometer exercise and monitored treadmill exercise will be used to classify participants into fitness categories and to explore the age relationships of biochemical and metabolic responses to exercise. Measurements of lung volumes and uniformity of pulmonary ventilation will be made to characterize the respiratory competence of the longitudinal studies participants.

Publications:

Borkan, G.A. and Norris, A.H.: Pattern profile approach to biological age in adulthood. J. Gerontol. In press.

Tzankoff, S.P. and Norris, A.H.: Age related differences in lactate distribution kinetics following maximal exercise. Eur. J. Appl. Physiol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00017-21 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Age Relationships of Body Composition, Nutrition and Physical Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.H. Norris	Chief, Human Performance Section	CPB NIA
	R. Andres	Chief, Clinical Physiology Branch	CPB NIA
	S.P. Tzankoff	Sr. Staff Fellow	CPB NIA
OTHER:	N.W. Shock	Scientist Emeritus	NIA
	J.D. Tobin	Medical Officer	CPB NIA
	V. Elahi	Epidemiologist	CPB NIA
	D. Elahi	Staff Fellow	CPB NIA
	R. Aamodt	Chief, Whole Body Counter Section	NM CC

COOPERATING UNITS (if any)
P.T. Davis, Dept. of Medicine, University of Buffalo, Buffalo, N.Y.
S.M. Garn, Ctr. for Human Growth & Development, University of Michigan, Ann Arbor

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.00	.75

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study of the interrelationships of body composition, nutrition and physical activity is a longitudinal study of aging. It provides a description of these characteristics for participants in the Baltimore Longitudinal Study. It provides opportunity to relate changes in these basic characteristics of the individual participants to changes in other biochemical, physiological and psychological measurements. A variety of non-invasive techniques are employed. They include the Behnke Anthropometric Index, skinfold thickness measurements, height, weight, twenty-four hour creatinine excretion, total body potassium determination, basal metabolism determinations, Garn X-ray fat thickness measurements, a diet diary, and an activity questionnaire. Previously, measures of total body density and total body water have been made in longitudinal studies participants. Body density corrected for differences in body water content have been compared with the Behnke Index and other conventional anthropometric indices (such as ponderal index).

PHS-6040
(Rev. 10-76)

Objectives: This project is designed to describe age differences and age changes in body composition, nutrition, and physical activity. Mechanisms of interaction of these functions and behaviors will be sought. The relationship of these measurements to other physiological, psychological and biochemical variables will be examined.

Methods Employed: Height, weight, and body circumferences of longitudinal study participants are obtained by standard anthropometric methods. Roentgenographic and anthropometric estimates of skeletal mass are combined with height, weight, and body circumferences to provide an estimate of body fat. Other estimates of fat include skinfold thickness measurements and fat thickness measurements from X-rays. Indices of lean body mass include: (1) basal metabolic rate determinations, (2) twenty-four hour urinary excretion of creatinine, (3) total body potassium, and (4) total body water and extracellular water determinations by indicator dilution. Nutrient intakes and activity calories are estimated from a diary and a self-administered questionnaire. All such measurements are repeated in the course of each subject's participation in the longitudinal program.

Analysis of the nutrition data followed the Schaie-Baltes model in order to separate the effect of aging from secular and cohort effects. The analytic objective was to determine how diet varies with age and how diet may have changed since the early 1960's. To answer these questions it is necessary to follow a group of men as they age to distinguish aging, secular and cohort effects on the nutritional variables of interest.

Food intakes from seven-day dietary records provided by 845 male participants were converted into nutrient intakes by trained dietiticians and a computer program based DOA Handbook 8 as updated for these purposes. The subjects selected for inclusion in this study met the following criteria. First, each subject had to have supplied at least three dietary records. It was felt that a minimum of three observations was necessary to validly show a subject's trend in diet over time. Four hundred eighty-nine (489) subjects satisfied this criterion. Secondly, the subject had to have a record in each of three time periods, 1961-65, 1966-70 and 1971-75 (called Epoch 1, Epoch 2, and Epoch 3, respectively). These 5-year periods represent equal divisions of the time span in which dietary diaries were completed, that is 1961-75. This criterion was intended to maximize the length of the study period and to insure that all subjects entered observation at the same time. Two hundred fifty-six (256) subjects met this criterion. Finally, one had to be able to select a record from each time period such that the three records were in three consecutive five-year age categories. This allowed us to group the subjects into age quintiles and to follow each initial age group as a cohort in the analysis. One hundred ninety-nine (199) men satisfied all three of these criteria, and they ranged in age from 25 to 82 at the time of their first record. In 9 of the quintiles (ages 35-80) there were at least 5 subjects per quintile and analyses are based on those 9 cohorts.

Major Findings: Details of a study of bone loss in participants in the Baltimore Longitudinal Study of Aging are reported in project no. Z01 AG 00022-03 CPB.

PHYSICAL ACTIVITY AND MUSCLE MASS IN WOMEN: Women participants in the Baltimore Longitudinal Study of Aging have been compared to men with respect to muscle mass and its relation to physical activity. Analysis of data on skeletal muscle mass (estimated by 24-h urinary creatinine excretion) in 146 women participants of the BLSA shows progressive and consistent age-related decrements. In the four decades spanning 35 to 75 years of age for which the data are most numerous, the older subjects' muscle mass was about 25% lower than that of the younger ones. This difference is similar to the 28% decrement found for men over the same age span. The consistency of decrements in muscle mass with age did not suggest any effect attributable to menopause, since women over the age of 50 years continued to decrease at rates similar to the young.

Comparisons between physically active women (at least 15 min of exercise per day) with sedentary women of similar age did not reveal muscle mass differences attributable to physical activity. These findings are also similar to those for men for whom even higher levels of habitual physical activity had been considered.

Basal oxygen consumption, which for the men was previously shown to decrease as a function of the age-related loss of skeletal muscle mass shows similar trends in women. In 101 female participants of the BLSA for whom paired observations of both skeletal muscle mass and basal oxygen consumption were available there was a statistically high association between these two measurements ($p<.001$). Basal oxygen consumption in women declined an overall 5.9 ml O_2/min/decade of life, a rate considerably lower than the mean of 8.2 ml/min/decade observed in men. However, the data for women presumed (on the basis of age) to be premenopausal show mean rates of decline of about 9 ml/min/decade while post-menopausal women had rates of decline of about 4/ml/decade. The lower rates for post-menopausal women may be a reflection of greater energetic demands associated with increased body fat deposition, a well known post-menopausal phenomenon, or disease processes which may also increase O_2 uptake as has been shown for men (see publication listed below).

Significance to Bio-Medical Research and the Program of the Institute: Nutritional deficiencies in the aged are known to be common and are generally attributed more to the socio-economic deprivation of this group than to biological or physiological aging effects. The volunteers in the Longitudinal Study Group are not a deprived group--it may be characterized as upper-middle class and has a very high educational level. It, therefore, offers a unique opportunity to study nutritional status under very favorable conditions. The nutritional effects of biological age per se may, therefore, be separated from what might be called "social aging."

Certain age changes in organ systems and various diseases are thought to be affected by diet, level of physical activity, and body composition. From the repeated assessment of these factors over time, it may be possible to determine their relative contributions to longevity and the maintenance of health and vigor in later life. Difficulties associated with obtaining retrospective estimates of eating habits, activity and body composition in the past make a prospective approach necessary for the collection of reliable information.

Proposed Course: Studies of diet, physical activity and body composition will continue. Data already collected will be further analyzed. Interactions of changes in body composition, food intake, food composition, kind and amount of physical activity, disease, and age will be examined. Specifically, body fat and lean body mass estimates, nutrient intakes and physical activity will be used in an analysis of risk of cardiovascular disease and of rate of aging of several organ systems.

Publications:

Tzankoff, S.P. and Norris, A.H.: Longitudinal changes in basal metabolism in man. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 45(4): 536-539, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00018-13 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Marital, Sexual and Social Factors in Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Martin	Sociologist	CPB NIA
OTHER:	S.M. Harman	Medical Officer	CPB NIA

COOPERATING UNITS (if any)
Baltimore City Hospitals

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.80	.60	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Systematic data concerning sexual, marital and social experience were obtained by structured interviews with males taking part in the Baltimore Longitudinal Study of Aging. During the year it was found, in collaboration with Dr. S.M. Harman, that frequency of sexual expression in 60-79 year-old respondents showed a small but significant relationship to level of serum testosterone and that levels of basal sex steroids remained largely unaffected by age. At present, an analysis is underway in which respondents with and without a history of coronary heart disease are being compared in relation to a variety of personal and behavioral experiential attributes, as revealed by interview, for evidence of etiologic processes.

PHS-6040
(Rev. 10-76)

Objectives: Present objectives are to: 1) determine whether coronary artery disease, and other pathology of unknown etiology, may be related to some aspect of marital, sexual or social experience, 2) explore the significance of a variety of physiologic and psychologic factors as potential correlates of the variation in levels of sexual functioning in older male respondents, and 3) conduct interviews with study members who have not been interviewed.

Methods Employed: In the course of his participation in the longitudinal program, each man is asked to contribute an interview concerning his history of marriage and sexual activity. In requesting interviews the present investigator describes study objectives in detail, provides assurance of confidence, and emphasizes the voluntary nature of such a contribution. Over the years the refusal rate has varied from 2 to 3 percent.

To facilitate communication and remembering and to insure systematic data collection, the questions asked are committed to memory by the investigator along with whatever categories are required for classifying responses. To help establish rapport and to generate information not other wise obtained, a number of aspects of occupational, educational, religious, military, and parental home experience are reviewed before introducing questions relating to marital adjustment and sexual conduct. The data obtained are believed to be of unusual quality because of the excellent mental status of respondents and their evident interest in this part of the program.

Major Findings: Perhaps the findings of greatest interest involving sexual data obtained during the year were those found by Drs. S.M. Harman and P.D. Tsitouras. In relating frequency of sexual expression to levels of plasma testosterone in males aged 60-79, they found a small but statistically significant relationship. However, they also found that levels of basal sex steroids were maintained with increasing age in spite of a steady decline in rate of sexual activity with age. These and other findings are described in Dr. Harman's report (project report number Z01 AG 00023-03 CPB).

A plan for last year was to initiate a broad search among physiologic and psychologic variables for evidence of correlation with sexual behavior. This effort had to be postponed due to the departure of H. Seideman who was an important collaborator in the endeavor. The objective of the inquiry was to pursue the finding that the decline in male sexual vigor with age is, in all likelihood, the product of the aging male's loss of ability to cognitively translate available visual stimuli into erotic arousal and thus sustain awareness of sexual need. As statistical and programming skills again become available, the appropriate analyses will again be pursued.

In consequence, it was decided to turn to the question of whether study members diagnosed as having definite or probable evidence of coronary artery disease (CAD) differed from age-matched controls in regard to various aspects of marital, sexual or social experience. Analyses appropriate to this question can now be undertaken because of the availability of diagnostic data on CAD, because death certificates are now available for most deceased study members allowing for additional assessment of CAD status, and becuase a sufficient number of interviewees have acquired diagnoses of definite CAD (N=138) or probable CAD (N-104) as to allow comparison with age-matched controls.

The variables to be considered in such comparisons number well over one-hundred, and range over many personal and behavioral attributes of the life history. These include measures pertaining to: quantity of coital and non-coital activity reported for various ages, number of coital partners and number of changes of marital status by 20, 40 and 60 years of age, types of sexual dysfunction, marital adjustment, and other experiential data in relation to farm residence, military service, parental-home relationship, and various indicators of emotional disturbance. As these data are compiled onto a series of tapes, it becomes a relatively simple matter to extend the analysis to include comparisons between respondents categorized according to history of hypertension, hyperlipidemia, diabetes, and other known risk factors for coronary disease.

Significance to Bio-Medical Research and the Program of the Institute: There are few specific hypotheses available to guide analysis because of the lack of prior investigation in this area of inquiry. Nevertheless, the dramatic defferences in mortality rates from all causes and from CAD between married and unmarried in vital data appear to warrent such investigation. Moreover, exploratory analysis of this kind would seem appropriate when they can be inexpensively carried out on data that are not likely to be duplicated for years to come.

Proposed Course: The immediate problem in pursuing this line of inquiry continues to be one of identifying subjects according to diagnostic status for each type of pathology and their respective age-matched controls. Once this is accomplished the remaining computations can be done on a routine basis.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00021-16 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Dermatoglyphics in: 1. Populations 2. Medicine 3. Aging 4. Families 5. Twins 6. Methodology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Plato	Geneticist	CPB NIA
OTHER:	D.C. Gajdusek	Chief, Lab. of Central Nervous System Studies	CNS NINCDS
	R. Garruto	Sr. Staff Associate	CNS NINCDS
	B.D. Bricker	Computer Specialist	CPB NIA

COOPERATING UNITS (if any)

See attached page.

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.50	.20	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project represents an ongoing joint collaborative effort, involving the WHO and other national and international biological laboratories to coordinate the collection, evaluation and interpretation of dermatoglyphic data. Specifically the objectives of this project are: 1) to study the distribution of dermatoglyphics among the various human populations (population dermatoglyphics); 2) to establish the dermatoglyphic frequencies in normal control samples (control dermatoglyphics); 3) to establish dermatoglyphic markers in various diseases (clinical dermatoglyphics); 4) to study the dermatoglyphics of the aged: 5) to study the genetics of dermatoglyphics; and, 6) to utilize dermatoglyphics as an added tool in twin diagnoses (twin dermatoglyphics).

PHS-6040
(Rev. 10-76)

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6. T. Kuberski, Epidemiologist
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14. B. Schaumann
    Neurology Section
    The Veterans Administration Hospital and
    The University of Minnesota
    Minneapolis, Minnesota

15. R. G. Schamschula
    The Institute of Dental Research
    The United Dental Hospital of Sydney
    Sydney, Australia

Project Description:

Objectives: This ongoing project represents an extensive collaborative effort, in conjunction with WHO and other national and international institutions, to study all aspects of dermatoglyphics. The specific objectives of this study are: (1) to establish the distribution of dermatoglyphic features in various populations, with special emphasis on primitive and other isolated groups in the South Pacific and other parts of the world. (2) To establish associations between dermatoglyphic features and specific clinical anomalies. (3) To study the dermatoglyphic frequencies in different age groups. (4) To investigate the genetic aspects of dermatoglyphics through family and twin data. (5) To improve and standardize the international methodology and nomenclature on dermatoglyphics.

The overall purpose of these studies is to utilize the dermatoglyphic markers in an effort to study the genetic structure of different populations, and to provide additional tools for studying the etiology of certain diseases.

Methods: Digital and palmar prints collected by different groups through various methods are sent to our laboratory for evaluation and interpretation of the results. We developed new methods and computer programs for studying and analyzing the dermatoglyphic data. These methods have been accepted and are utilized by other laboratories here and abroad.

Major Findings: Even though the dermatoglyphics of the same individual remain unchanged throughout life (longitudinally), cross-sectional comparisons indicated a decreasing frequency of palmar patterns and increasing transversality of palmar ridges at the older ages. In the area of population dermatoglyphics, we completed a preliminary evaluation on 75 isolates from Australasia (out of the total of 90 studied from this region) and the results were presented at the XIV International Congress of Genetics. These data will be incorporated into the overall genetic mapping of these isolates. On the island of Guam we found no significant dermatoglyphic differences between patients with Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex and non-affected Chamorro controls.

Significance to Bio-Medical Research and the Program of the Institute:
1) To utilize dermatoglyphics as an added genetic marker in the evaluation of the longitudinal study participants and the overall study of aging.
2) To offer another tool in investigating the etiology of clinical entitites.
3) To provide additional genetic information to the study of primitive isolates.
4) To assist in compiling internationally standardized dermatoglyphic data which would be used effectively in studies of disease associations.

Proposed Course: To continue this project by evaluating the collected data and prepare reports for publication. To obtain additional clinical and population data from patients and normal controls of both sexes and all age groups.

Publications:

Plato, C.C.: Dermatoglyphics and their significance in aging studies. Geriatrics Digest (Medicine), pp. 6-7, February 1979.

Pollitzer, W. and Plato, C.C.: Anthropology and Dermatoglyphics. In Wertelecki, W., Plato, C.C. and Bergsma, D. (Eds.): Dermatoglyphics--50 Years Later. New York, Alan Liss Publishers. In press.

Garruto, R.M., Plato, C.C., Hoff, C.J., Newman, M.T., Gajdusek, D.C. and Baker, P.T.: Characterization and Distribution of Dermatoglyphic Features in Eskimo and North, Central and South American Indian Populations. In Wertelecki, W., Plato, C.C. and Bergsma, D. (Eds.): Dermatoglyphics--50 Years Later. New York, Alan Liss Publishers. In press.

Editor: Wertelecki, W., Plato, C.C. and Bergsma, D. (Eds.): Dermatoglyphics--50 Years later. New York, Alan Liss Publishers. In press.

Plato, C.C., Garruto, R.M., and Gajdusek, D.C.: The distribution of dermatoglyphic traits among the Australasian population. Proceedings of the XIV International Congress of Genetics, Moscow, U.S.S.R. In press.

Schaumann, B., Plato, C.C. and Wertelecki, W.: Selection of Controls in dermatoglyphics. Proceedings of the XIV International Congress of Genetics, Moscow, U.S.S.R. In press.

Wertelecki, W. and Plato, C.C.: The world distribution of simian and Sydney palmar flexion creases and their significance in medical disorders. Proceedings of the XIV International Congress of Genetics, Moscow, U.S.S.R. In press.

Plato, C.C.: Book Review: Dermatoglyphics in medical disorders by Blanka Schaumann and Milton Alter. Springer-Verlag, N.Y. 1976. Human Biology, 51:101-102, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00022-03 CPB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Investigations of Osteoarthritis and Bone Loss

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Plato	Geneticist	CPB NIA
	A.H. Norris	Chief, Human Performance Section	CPB NIA
OTHER:	D.C. Gajdusek	Chief, Lab. of Central Nervous Systems Studies	CNS NINCDS
	R.M. Garruto	Sr. Staff Associate	CNS NINCDS
	W.W. Greulich	Dept. of Anatomy, Stanford U.	

COOPERATING UNITS (if any)

Tecumseh Michigan Community Health Study
The University of Michigan, Ann Arbor, Michigan

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Human Performance Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.90	.60	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Osteoarthritis and bone loss are the two principal age related changes of the human skeleton. Even though these changes are considered inherent to aging, they may result in incapacitating ailments. The advanced cases of osteoarthritis (degenerative joint disease) produce severe restriction of movement associated with pain. Advanced bone loss may result in osteoporosis and frequent bone fractures. Most prominent are vertebral compression fractures and fractures of the femoral neck.

This project deals with the epidemiological, genetic and longitudinal aspects of osteoarthritis and bone loss among (1) the participants of the Baltimore Longitudinal Study, (2) in a sample of normal Guamanians (Chamorros), and (3) among patients afflicted with Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam.

Objectives: The objectives of this study are: 1) To study the cross-sectional and longitudinal changes in bone measurements and bone mineral content at different parts of the skeletal system and to identify factors which may affect them. 2) To evaluate the cross-sectional longitudinal and bilateral aspects of osteoarthritis in the digits and the vertebral column. 3) To investigate the longitudinal, familial and secular changes in bone measurements and bone density by examining Guamanian x-rays of the same individuals collected 33 years apart. 4) To study a) the possible differences in bone density between patients with Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex (ALS/PD) of Guam and a non-affected Guamanian sample and b) the effect of long term immobilization, due to paralysis, on bone mineral content. (See project Z01 AG 00028-03.) 5) To investigate possible relationship between lateral functional dominance and osteoarthritis and bone measurements.

Methods Employed: Radiographs were graded for osteoarthritis for each of the proximal and distal interphalangeal joints utilizing the internationally accepted grading system of J. H. Kellgren. Grades 0 and 1 were considered negative (normal), and grades 2, 3 and 4 were considered as having osteoarthritis (affected). Bone measurements were made for the total width, medullary width and the length of the second metacarpal bone. From these measurements we calculated the combined cortical thickness, the cortical area, the cortical area index and the cortical volume. The cross-sectional study was based on both the left and the right hands. The longitudinal study included participants with at least three x-rays; only left hands analyzed. Lateral functional dominance was established through a series of tests involving gross as well as fine manipulations of the hands. Foot and eye preferences were also ascertained as well as grip strength. In 1947, Dr. William W. Greulich collected hand x-rays and conducted antropometric studies on several hundred Guamanian children, ages 5-17 years. These children lived through all or part of Japanese occupation, a period marked by severe nutritional deprivation. Bone density measurements will be made from these x-rays.

Major Findings: A) Osteoarthritis: The prevalence of osteoarthritis of the hand varies from one digit to the other. Osteoarthritis is considerably more prevalent and appears in a more severe form in the distal than the proximal interphalangeal joints regardless of digit or age group. Longitudinally studied, osteoarthritis is found to be a relatively slow process, with a maximum average increase of 1 grade per individual in an interval of 12 to 16 years between visits as seen in the distal interphalangeal joints. The rate of degeneration in the proximal interphalangeal joints is much lower. The progress of joint degeneration in the distal interphalangeal joints (longitudinally evaluated) is directly related to age. This is not always seen in the proximal interphalangeal joints. B) Bone Density and Bone Measurements: There is progressive bone loss with age resulting from resorption at the endosteal surface. After adulthood, medullary diameter is increased with age, while total periosteal diameter and length do not change significantly. Physical stress was found to be correlated with total periostial diameter but was not associated with medullary diameter. Since total bone diameter and length do not change with age after adulthood, the association between physical strength and bone size must have its effect before adulthood is attained. Bilateral comparisons show consistently higher bone size and mass in the right hands. Bilateral differences in total width, length, total area and cortical area are significant among the right hand dominant and non-significant among the left hand dominant participants. Regardless of hand dominance there were no bilateral differences in medullary width. These results suggest an inherent tendency of the right second metacarpal to have more bone than the left regardless of hand dominance. Differential stress due to hand dominance will increase the bilateral differences among the right handed and reduce it in the left handed. Preliminary results indicate that ALS patients have lower mineral content than non-affected Guamanians.

Significance to Bio-Medical Research and the Program of the Institute: Loss of bone tissue and deterioration of the joints are significant causes of disability and death in older people. Determination of the existence and degree of disease in relatively well ambulatory people provides a unique opportunity to compare bone loss and degeneration with other characteristics of these people. Nutrient intakes, activity levels and muscle strength among other things may be compared.

Proposed Course: To finish the evaluation of the collected data and publish the results. To continue the collection of bilateral x-rays from the male as well as from the female participants of the Baltimore Longitudinal Study. Now that the prevalence and rates of change in bone loss and joint degeneration have been described, comparisons of these processes with other characteristics and processes in these participants will be undertaken. Such things as nutrient intakes, medications, physical activity and muscle strength will be considered. To expand the data on the ALS/PD patients, suspected patients and non-affected Guamanian controls. To study the cross-sectional and longitudinal aspects of bone density and osteoporosis in the thoracic spine and the ulna and radius of the hand, and to compare the relative severity and rate of bone loss at different skeletal sites. To ascertain possible associations between the two types of bone changes (osteoarthritis and bone loss) and certain diseases as well as physiologic and anthropometric variables. To investigate the relationship of bone loss to blood levels of calcitonin parathyroid hormone and its subchains and other hormones, as well as vitamin D, calcium, phosphorus, flouride and magnesium. To compare bone density values obtained through x-rays with those of other methods.

Publications:

Plato, C.C. and Norris, A.H.: Osteoarthritis of the hand: Age specific joint-digit prevalence rates. Am. J. Epidemiol. 109:169-180, 1979.

Plato, C.C. and Norris, A.H.: Osteoarthritis of the hand: Longitudinal studies. Am. J. Epidemiol. In press.

Plato, C.C., Wood, J., and Norris, A.H.: Bilateral asymmetry in bone measurements of the hand and lateral hand cominance. Am. J. Phys. Anthrop. In press.

Plato, C.C. and Norris, A.H.: Bone measurements of the second metacarpal and grip strength. Human Biology. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00028-03 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Epidemiologycal and Genetic Studies of Amyotrophic Lateral Sclerosis/ Parkinsonism Dementia (ALS/PD) Complex of Guam

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Plato	Geneticist	CPB NIA
OTHER:	D.C. Gajdusek	Chief, Lab. of Central Nervous Systems Studies	CNS NINCDS
	R.M. Garruto	Sr. Staff Associate	CNS NINCDS

COOPERATING UNITS (if any)
C & F Research Center, NINCDS

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: .60	PROFESSIONAL: .30	OTHER: .30

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is: 1) to investigate the genetic and epidemiological factors contributing to the very high incidence of Amyotrophic Lateral Sclerosis and Parkinsonism Dementia (ALS/PD) on Guam, 2) to evaluate the distribution of the various established genetic and anthropological markers among the normal Guamanian population and compare them with those of the ALS/PD patients, and 3) to ascertain the effects of immobilization due to paralysis on bone density.

Objectives: Our involvement in this multidisciplinary project has the following objectives: 1) to identify the epidemiological variables which contribute to the very high incidence of Amyotrophic Lateral Sclerosis/Parkinsonism Dementia (ALS/PD) Complex of Guam, 2) to ascertain the possible changes in the incidence of ALS/PD, 3) to determine the extent of genetic involvement in the etiology of the disease, 4) to study the distribution of several established genetic markers such as blood groups, serum proteins, red cell enzymes and dermatoglyphics in the normal Guamanian (Chamorro) population to be used as controls in comparisons with patients, 5) to determine the prevalence of osteoarthritis, osteoporosis and other bone diseases in Chamorro ALS/PD patients and non-affected controls, and 6) to study bone growth and development of normal Guamanian children and adolescents in a 33 year follow-up study (See project #Z01 AG 00022-03 CPB).

Methods: Eighteen years ago we established two approaches towards the study of familial and genetic aspects of ALS/PD. First, the patient control registry panels, which consist of 136 patients, 136 individually matched controls and their respective sibs, parents, offspring and spouses. Second, collection of complete pedigree data for each patient as well as complete genealogy of all individuals born in Umatac, the village with the highest prevalence of ALS/PD. Both of these studies are presently being updated and the information is evaluated. Blood group and dermatoglyphic data were evaluated by the standard methods. X-rays from patients and controls are collected and evaluated using the methods described in project #Z01 AG 00022-03 CPB.

Major Findings: The epidemiological and genetic data, as well as osteoarthritis and bone loss data are presently being analyzed. Blood group and dermatoglyphic comparisons between patients and controls are completed and were reported at the XIV International Congress of Genetics (Moscow 1978). There were no significant dermatoglyphic differences between ALS/PD patients and non-affected controls. There were, however, significant differences between the two samples in the frequencies of ABO, Rh, MNS and Diego blood group systems.

Significance to Bio-Medical Research and the Program of the Institute: The ultimate goal of this multidisciplinary program is not only to elucidate the etiology of Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam, but also to provide a model for studying other neurological diseases and dementias which are for the most part diseases of old age. It will also provide information on the effect of immobilization due to paralysis on bone mineral content (bone loss).

Proposed Course: To reevaluate the last updating (February 1977) of the patient-control registries, pedigrees. Also to review all the epidemiological and genetic data accumulated during the past twenty years; to finish the computations on the bone density and osteoarthritis data of patients and controls and prepare reports for publication.

Publications:

Garruto, R. M., Plato, C. C. and D. C. Gajdusek: The dermatoglyphics and blood group frequencies in patients with Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam and non-affected Guamanian controls. Proceedings of the XIV International Congress of Genetics, Moscow, U.S.S.R. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00010-06-CPB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Echocardiographic Assessment of the Left Ventricle and Mitral Valve in Aging Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Fleg	Staff Cardiologist	CPB, NIA
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
Other:	None		

COOPERATING UNITS (if any)

F. C. P. Yin, Div. of Cardiology, Dept. of Medicine, Johns Hopkins Medical Institutions

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.8	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Earlier echocardiographic work from this laboratory has shown that the left ventricular thickness and aortic root diameter increase as a function of age whereas cavity size, ejection fraction and velocity of shortening are unchanged and mitral valve closure slope is reduced. Since this initial project was limited to men with a maximum blood pressure of 140/90, we have extended this study to women and extended the blood pressure range for entrance into the study of both sexes across a spectrum of ages (18-95).

A research contract involving the Cardiology Division of Johns Hopkins Hospital to evaluate cardiac anatomy and function at rest and during exercise began on 1 November 1977 and will extend for three years. Two-dimensional exercise echocardiography and thallium^{201} myocardial perfusion scanning are being employed. This is discussed in the appendix to this report.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: (1) Echocardiography is an ideal non-invasive method for assessing age-related changes in cardiac anatomy and function. We hope to determine the pattern of these changes in normal women and compare the results to those of normal men of the same ages. Hypertension is known to induce thickening (hypertrophy) of the left ventricular walls. Echocardiography will be utilized to determine the extent of this hypertrophy, and whether increases in blood pressure that occur with age are correlated with thickening of the left ventricle.

(2) Non-invasive techniques are employed in man to measure the function of the left ventricle while it is subjected to a known amount of stress. The rationale is that diseased or failing hearts may appear to function normally when not subjected to undue stress, but reveal abnormalities when subjected to even a moderate degree of stress. Previous animal experiments from this laboratory have demonstrated that the aged but otherwise healthy stressed heart has certain functional impairments. comparable information in man is lacking. Utilizing modern techniques, it is possible to examine this question in man. By comparing the performance of normal (by standard criteria) young and aged hearts both unstressed and during the imposition of a controlled degree of stress, one is able to assess the degree of impairment in heart function due to the aging process.

Methods: Echocardiograph assessment of the aging heart includes two major projects. (1) Normal men and women in the Baltimore Longitudinal Study and those with uncomplicated hypertension receive resting echocardiograms. Left ventricular wall thickness, systolic and diastolic dimensions and rate of endocardial shortening, mitral valve closure rate, plus aortic root and left atrial dimensions are measured.

(2) The second study utilizes the echocardiogram to measure left ventricular function during imposition of a stress. A predetermined increase in peripheral blood pressure is induced sequentially by isometric handgrip exercise or by infusion of an epinephrine-like drug (phenylephrine). Measurements are made before and after temporary blockade of the sympathetic nervous system achieved by infusing the beta-adrenergic blocking drug propranolol. Electrocardiograms and blood pressure are recorded simultaneously with the echocardiogram. Left ventricular function is assessed by measuring changes in the diastolic and systolic dimensions and velocity of shortening of the endocardium during the various interventions.

Major Findings: (1) The results in normal men were presented by Gerstenblith et al., Circulation 56: 272-278, 1977. The data on women and hypertensives are currently being gathered and analyzed.

(2) The results of Project II demonstrate that when beta-adrenergic tone is eliminated, the left ventricular cavity dilates in response to phenylephrine more in the aged than in the adult population.

Significance to Biomedical Research and the Program of the Institute: Information concerning the anatomic and functional aging changes of the normal human heart is critical to understanding the aging process of the cardiovascular system. Similar information is needed in aged "hypertensives" (over 10% of the population) to examine the effects of hypertension on the heart and their interrelationships with the aging process.

Proposed Course: The current project will be continued at least through the next fiscal year.

Publications:

Yin, F. C. P., Raizes, G. S., Guarnieri, T., Spurgeon, H. A., Lakatta, E. G., Fortuin, N. J., and Weisfeldt, M. L.: Age-associated decrease in ventricular response to haemodynamic stress during beta-adrenergic blockade. Brit. Heart J. 40: 1349-1355, 1978.

Lakatta, E. G.: The Cardiovascular System and Aging. In Busse, E. W. (Ed.): Cerebral Manifestations of Episodic Cardiac Dysrhythmias. Princeton, Excerpta Medica, 1979, pp. 51-59.

Appendix

Contract Number: N01-AG-7-2129

Contract Title: Non-invasive Assessment of Cardiac Structure and Function in Aging Men and Women

Contractor: Johns Hopkins University, Baltimore, Maryland
Investigators: G. Gerstenblith, Asst. Prof. of Medicine
M. L. Weisfeldt, Dir., Div. of Cardiology
J. Weiss, Asst. Prof. of Medicine
L. Becker, Asst. Prof. of Medicine

Money Allocated: $368,605.00

Objectives: Two-dimensional echocardiography is a new technical development which allows determination of cardiac anatomy and function in much the same manner as routine echocardiography, with the important addition that an entire plane of the heart can be visualized at once rather than a simple "ice pick" view, allowing greater accuracy in the determination of heart chamber shape, size and function. The goal is to examine 750 subjects from the Baltimore Longitudinal Study over a three year period (ages 18-95) during rest and maximal semi-supine bicycle exercise to determine age related differences in regional and global myocardial function.

Thallium^{201} myocardium imaging allows non-invasive assessment of regional left ventricular blood flow and will be used to determine the incidence, severity and prognostic implications of ischemic heart disease in Baltimore Longitudinal Study participants. The predictive values of this technique will be compared to that of stress electrocardiography and two-dimensional echocardiography. About 150 new subjects will be studied for each of three years. In year 5 the subjects in year 1 will be retested.

Methods: Subjects perform maximal graded semi-supine bicycle ergometry and simultaneous ECG and blood pressure monitoring every minute. Two-dimensional echograms are made in longitudinal and cross-sectional views of the left ventricle at rest, maximal exercise and during recovery with regard to the following echocardiographic indices (1) myocardial mass, (2) left ventricular chamber size, (3) mean and maximal velocity of fiber shortening and lengthening, (4) percent change plus mean and maximal velocities of regional left ventricular systolic thickening and diastolic thinning, (5) percent, mean and maximal rates of change in regional left ventricular radius and (6) duration of left ventricular ejection and filling.

Thallium imaging is performed in four views following peak exercise of a multi-stage maximal treadmill test. Subjects found to have perfusion defects after stress are re-imaged in 2 hours (without further nuclide injection) to determine whether there is any "filling in" of the defect.

Major Findings: To date, approximately 200 subjects have undergone each of the two procedures. A preliminary analysis of the echocardiographic data is in progress.

Thallium scanning has shown an increasing rate of positivity with age both in subjects with known coronary heart disease as well as in asymptomatic individuals. A high percentage of asymptomatic subjects, especially women, have shown ischemic exercise electrocardiograms (ECG) with normal thallium scans, suggesting a "false positive" exercise ECG.

Significance to Biomedical Research and the Program of the Institute: Two-dimensional echocardiography will allow detailed non-invasive analysis of regional and global cardiac wall motion in normal adults representing eight decades and in subjects with coronary artery disease. Such information should help to define early pathological changes in cardiac muscle function.

Thallium^{201} myocardial imaging will permit the non-invasive detection of coronary disease in a normal population. Periodic repeat examinations will allow the calculation of incidence of new disease as well as progression rates in subjects with manifest disease. Such information was previously available in coronary arteriography, an invasive, costly and more dangerous procedure.

Proposed Course: Both two-dimensional echocardiography and thallium ^{201} myocardial scanning should prove valuable tools for longitudinal assessment of cardiac wall motion and myocardial perfusion respectively and should allow the response to numerous medications and physiological interventions to be determined, both in normals of various ages and in patients with heart disease.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00024-03 CPB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Response to Cardiac Glycosides in Senescent Dogs, Rats, and Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	H. A. Spurgeon	Physiologist	CPB, NIA
	J. L. Fleg	Staff Cardiologist	CPB, NIA
Other:	J. P. Froehlich	Medical Officer	LMA, NIA

COOPERATING UNITS (if any)

T. Guarnieri, Dept. of Medicine, Johns Hopkins Medical Institutions
G. Gerstenblith, Div. of Cardiology, Dept. of Medicine, Johns Hopkins Medical Institutions

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.85	.80	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigation of the role of aging on the: (1) direct effect of cardiac glycosides in isolated cardiac muscle from rats; (2) effect of cardiac glycosides on electrophysiology and mechanical performance in intact dogs; (3) effect of cardiac glycosides and Na^+-K^+ ATPase inhibition in cardiac muscle from both rodent and canine species; and (4) effect of age on ouabain infusion in normal man.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The objectives of this study are to characterize the effect of age response to cardiac glycosides both in the intact organism and in isolated heart muscle.

Methods: (1) Intact dogs.

(a) Unanesthetized awake dogs are given rapid bolus injection of acetylstrophanthidin. This is a rapid acting glycoside. Doses are repeated in 5 μg/kg increments each 30 minutes until the end point of the study - ventricular tachycardia ensues. The dose required to produce this toxic effect is compared in both young adult and senescent beagles. Serum levels of the drug are monitored prior to each dose.

(b) Anesthetized dogs are instrumented with a catheter tipped micro-monometer placed in the left ventricle and a pacing catheter inserted into the right ventricle. The dose response curve obtained in a given dog in the awake state is repeated in the anesthetized state. The inotropic response of pressure and rate of pressure development are measured at a given paced rate; in addition toxic effects on the ECG are simultaneously measured.

(c) Beta-blockade. On a third day (nonsuccessive), part (b) is repeated in the presence of propranolol, a beta -adrenergic blocking drug. Thus in a given dog, electrical responses are measured in the awake state, and both electrical and mechanical responses are measured in the anesthetized state.

(2) Isolated muscle. Trabecular muscles are isolated from hearts of the rat and the response to ouabain is measured and compared to the response to other inotropic interventions (increased both calcium and paired stimulation).

(3) Ouabain inhibition of Na^+-K^+ ATPase is measured in membrane preparation from dog and rat hearts.

Major Findings: (1) Results indicate that there is no age difference in the dose of ACS necessary to induce ventricular tachycardia in the awake dog. There is no age difference in the toxic threshold to ACS during anesthesia. The peak inotropic response, however, is twofold greater in the adult than in the senescent beagle. This age difference in inotropic response to ACS persisted during beta-blockade. These data have been presented at the Scientific Sessions of the American Heart Association, October 1978 (Guarnieri et al., Circulation 58:

II-147, 1978). The manuscript has also been accepted for publication (vide infra).

(2) In isolated rat cardiac muscle, there is a marked significant decrement in the inotropic response to ouabain, while no age difference exists in response to calcium or paired pacing. There is no age difference in the ouabain inhibition of Na-K ATPase either in the dog or in the rat. This suggests that either the number of receptors or steps intermediate between the receptor and effector are altered with age. These data have been published (vide infra).

Significance to Biomedical Research and the Program of the Institute: The age difference demonstrated in the direct effect of ouabain on isolated cardiac muscle indicate that the number of sarcolemmal receptor sites or ion exchange pumps may be altered with age. Since cardiac glycosides are widely used in treatment of heart failure, the demonstration of an age difference in response to these agents would be of obvious clinical import.

Proposed Course: The feasibility of measuring the response to ouabain infusion as a function of age in normal man has been determined. The effect of an intervenous bolus of ouabain, a short-acting digitalis glycoside, is being studied in young and elderly men. Inotropic response is measured by means of echocardiography and systolic time intervals. To date, ten subjects have been studied; results are currently being analyzed.

Publications:

Gerstenblith, G., Spurgeon, H. A., Froehlich, J. P., Weisfeldt, M. L., and Lakatta, E. G.: Diminished inotropic responsiveness to ouabain in aged rat myocardium. Circ. Res. 44: 517-523, 1979.

Guarnieri, T., Spurgeon, H. A., Froehlich, J. P., Weisfeldt, M. L., and Lakatta, E. G.: Diminished inotropic response but unaltered toxicity to acetylstrophanthidin in the senescent beagle. Circulation (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00026-03 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Age-Associated Alterations in Response to Catecholamines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	C. Filburn	Staff Fellow	LMA, NIA
	E. Beard	Chemist	CPB, NIA
	G. Roth	Research Chemist	CPB, NIA
Others:	None		

COOPERATING UNITS (if any)
T. Guarnieri, Department of Medicine, Johns Hopkins Medical Institutions

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.9	.1

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous work from this laboratory has demonstrated that when compared to the young adult rat myocardium, that from aged rats exhibits a diminished inotropic response to catecholamines. In addition, the response to these agents in both the canine model and in man is diminished in senescence, compared to that observed in mature adults. The present work includes measurements of cyclic nucleotide levels, and protein kinase activation in perfused rat interventricular septa which have been stimulated with isoproterenol and dibutyryl cAMP, and in which the mechanical response to these agents has been quantitated. β-receptor number and affinity as well as cAMP-protein kinase stimulation of Ca^{++} accumulation in isolated sarcoplasmic reticulum have also been measured.

Project Description:

Objectives: (1) To measure the inotropic effect of catecholamines in the perfused interventricular septum of the rat with measurements of cAMP and protein kinase activation, in the same tissue. (2) To measure the number and affinity of β-receptors in hearts from adult and aged rats. (3) To measure the stimulation of Ca^{++} accumulation in sarcoplasmic reticulum from adult and aged rat hearts by protein kinase and cAMP.

Methods: (1) The rat interventricular septum is cannulated and perfused and force production in a control steady state is measured. The enhancement of force production in response to infused isoproterenol and dibutyryl cAMP is then measured. When the mechanical response has peaked, the septum is quick frozen in liquid nitrogen and analyzed for cAMP, protein kinase activity, protein kinase activation ratio, and phosphoproteinphatase, all of which determine the extent of intracellular phosphorylation and thus determine the magnitude of the contractile response. (2) β-receptors are measured by the method of Alexander et al., Proc. Natl. Acad. Sci. USA 72: 1564-1568, 1975. (3) cAMP protein kinase stimulation of sarcoplasmic reticulum preparations (Harigaya and Schwartz, Circ. Res. 25: 781-794, 1969) was measured as described by Tada et al., J. Biol. Chem. 249: 6174-6180, 1974.

Major Findings: The major finds of (1) and (2) are summarized in the abstract of the paper presented at the American Federation for Clinical Research, May 1979 (Guarnieri et al., Clin. Res. 27: 172A, 1979). Studies from our laboratory indicate that the contractile response to paired pacing or increased Ca^{++} is not altered in cardiac muscle from the senescent rat, but the response to catecholamines is diminished compared to the adult. This study investigated whether alterations in β-receptors, stimulation of cAMP, or activation of cAMP-dependent kinase could explain this age difference in contractile response. The maximum rate of force development (dF/dt) was measured in interventricular septa from 9 senescent (S, 24-27 mo) and 6 adult (A, 6-9 mo) rats perfused with Krebs buffer, pH 7.4, at 29°C and paced at 80/min. Control dF/dt was not different: A = 65.7±3.5 gm/sec; S = 75.9±4.9 gm/sec. Peak response to isoproterenol (Iso, $5X10^{-7}M$) was greater in A than S: A = 246.3±16% control, S = 202.7±8.3% control, $P<.03$. These septa were frozen and assayed for cAMP and cAMP-dependent protein kinase activation ratio (PKAR), measured as the ratio of kinase activity in the absence and presence of 2 μM cAMP. Control cAMP (A = .343±.02, S = .356±.01 pm/mg wet wt) and PKAR (A = .191±.04, S = .193±.02) measured in septa not exposed to Iso were not age related. At peak Iso, cAMP and PKAR were increased to a similar extent in both age groups: cAMP = .617±.03

in A, and .635±.01 pm/mg wet wt in S; PKAR = .439±.02 in A, and .434±.02 in S. Neither the number of alprenolol binding sites (A = 34.8±1.5, S = 39.0±5.3 fm/mg protein), nor the affinity (A, kd = 4.3±0.7 nM; S, kd = 5.8±1.1 nM) were age related. These data suggest that the mechanism for the diminished inotropic response to Iso in S is distal to cAMP-dependenct kinase activation, possibly at the level of phosphorylation of the sarcolemma and/or sarcoplasmic reticulum. These results in manuscript form have been submitted for publication.

(3) No age difference was observed in the increase in Ca^{++} accumulation velocity when in the presence of cAMP and protein kinase over a range of concentrations. This, as well as the identical shortening of twitch duration in both age groups, suggests that age differences in phosphorylation of sarcoplasmic reticulum is not the mechanism for the diminished response to β-adrenergic stimulation.

Significance to Biomedical Research and the Program of the Institute: The decrease response to all forms of stress in senescence versus adulthood - exercise, hypoxia, etc. may be explained at least in part by the diminished response to catecholamines, the physiological mediators of the stress response. Since the diminished stress response is one of most notable changes that occurs in an aged individual, studies to elucidate the mechanism of the altered response are of major import. These studies require collaboration between molecular biologist and physiologists and help establish an interface between these disciplines. This interface is a keystone in further studies in biomedical research.

Proposed Course: To measure Ca^{++} influx, in response to catecholamines, as measured by changes in slow inward Ca^{++} current as reflected in transmembrane action potentials in cardiac muscle from adult and senescent rats. A diminished increase in slow inward current in response to β-adrenergic stimulation appears to be the likely explanation for the diminished contractile response.

Publications:

Yin, F. C. P., Spurgeon, H. A., Greene, H. L., Lakatta, E. G., and Weisfeldt, M. L.: Age-associated decrease in heart rate response to isoproterenol in dogs. Mech. Ageing Dev. 10: 17-25, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00029-02 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Efficacy of Chronic Digoxin Therapy in Stable Congestive Heart Failure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Fleg	Staff Cardiologist	CPB, NIA
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA

Other: None

COOPERATING UNITS (if any)
S. H. Gottlieb, Div. of Cardiology, Dept. of Medicine, Baltimore City Hospital
Div. of Chronic Medicine, Dept. of Medicine, Baltimore City Hosp.

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Despite the nearly universal employment of digitalis in the treatment of chronic congestive heart failure (CHF), there is no objective documentation of its long-term efficacy. The current project is designed to evaluate, in a double blind crossover fashion, the effects of maintenance digoxin therapy in chronic stable CHF, utilizing clinical findings, chest radiography, echocardiography and systolic time intervals to assess baseline left ventricular function non-invasively and maximal exercise testing to determine aerobic work capacity.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: For some two hundred years, digitalis glycosides have been the cornerstone of therapy for CHF. The low toxic therapeutic ratio of digitalis results in the development of toxicity in 20-25% of patients and death in up to 20% of patients developing toxicity. Despite this universal employment of digitalis in the treatment of CHF, there is no objective documentation of its long-term efficacy. The current project utilizes several non-invasive parameters of cardiac function to assess the effects of maintenance digoxin therapy in stable CHF in a double blind crossover manner.

Methods: Patients in the Baltimore City Hospital Cardiology Clinic and the Chronic Hospital who have been on the same CHF medical regimen for at least 3 months will be studied. After a baseline clinical history and physical exam, each patient will receive a resting echocardiogram, systolic time intervals (STI), chest x-ray, and when possible, a maximal stress test. He will then be randomly assigned in a double blind fashion to either digoxin or placebo for 3 months and the studies repeated at the end of this period. Crossover will then occur and the testing repeated again 3 months later.

Major Findings: Thus far, none of the 18 patients withdrawn from digitalis has required urgent reinstituting of digoxin. No difference was found in symptoms or clinical signs including resting heart rate, weight, and cardiothoracic ratio between digoxin and placebo regimens. The velocity of circumferential fiber shortening (V_{cf}) and the fractional shortening of the minor semi-axis (FS) determined echocardiographically at rest, the ratio of pre-ejection period and ejection time (PEP/LVET) as well as the duration of maximal treadmill exercise (TM_{max}) while on placebo and digoxin were not different by paired t test below:

	PEP/LVET	FS	V_{cf}	TM_{max}
Digoxin	0.46±0.03	0.27±0.04	1.02±0.13	7.3±0.9
Placebo	0.45±0.02	0.25±0.04	0.91±0.13	7.5±0.8
N	17	10	10	8

Thus in these patients, who are representative of a large segment of all those treated with cardiac glycosides, digoxin has no demonstrable long-term effect on either clinical status or indices of cardiac muscle and pump function.

Significance to Biomedical Research and the Program of the Institute: If maintenance digitalis can be discontinued without ill effect in a sizable percentage of patients with stable chronic CHF, many episodes of toxicity (and death) could be avoided and medical expenses reduced. It should be noted that a sizable portion, if not the majority, of persons in whom digitalis preparations are prescribed are over 60 years of age.

Proposed Course: These non-invasive techniques for assessing cardiac function will continue to provide valuable objective parameters for evaluating the response to digitalis therapy in cardiac patients.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00030-01 CPB (Formerly Z01 AG 00009-04 CPB & Z01 AG 00025-02 CPB)

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Altered Relaxation and Stiffness in the Senescent Rat Heart: Relation to Hypertrophy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Spurgeon	Physiologist	CPB, NIA
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	E. Beard	Chemist	CPB, NIA
Other:	None		

COOPERATING UNITS (if any)
F. C. P. Yin, Div. of Cardiology, Department of Medicine, Johns Hopkins Medical Institutions

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

With advancing age, several characteristic alterations occur in the cardiovascular system. One of the most commonly described changes in senescence is a moderate degree of myocardial hypertrophy in both animals and in man. Other alterations seen in the senescent hearts are a prolonged time course of relaxation (Weisfeldt et al., Am. J. Physiol. 220: 1921-1927, 1971; Lakatta et al., J. Clin. Invest. 55: 61-68, 1975), a diminished rate of sarcoplasmic reticulum calcium accumulation (Froehlich et al., J. Mol. Cell. Cardiol. 10: 427-438, 1978) and an increased active dynamic stiffness in senescent compared with mature heart muscle (Spurgeon et al., Am. J. Physiol. 232(4): H373-H380, 1977) and intach hearts (Templeton et al., Circ. Res. 44: 189-194, 1979). Although the extent to which these functional alterations seen with senescence are related to the concurrent hypertrophy is unknown, a relationship is suggested since prolonged contraction duration, diminished sarcoplasmic reticulum function, and increased stiffness have been observed in experimental models of hypertrophy.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The goals of this study were (1) to determine the extent of hypertrophy and its relationship to alterations in relaxation and stiffness as a function of age in the rat model, (2) to create a mechanical overload hypertrophy of the same extent in adult rats to delineate the resulting functional alterations, and (3) to ascertain whether or not the alterations due to experimental hypertrophy are qualitatively and quantitatively analogous to the changes seen in the normal hypertrophied senescent heart.

Methods: (1) Heart weight and heart weight to tibial length ratios were measured in adult rats ages 6, 15 and 24 months of age to determine the extent of myocardial hypertrophy. (2) Mechanical parameters of the isometric twitch were studied in muscle isolated from hearts of rats of the ages noted above. (3) Aortic banding was implemented in middle-aged rats in order to induce hypertrophy of the same extent found in the senescent heart.

Major Findings: Selective LV hypertrophy of about 14% in the senescent compared to adult rat heart was found when tibial length, which is independent of changes in body weight, was used as the reference. Trabecular muscles from senescent hearts demonstrated a prolongation of contraction duration, an increase in the slope (both in contraction and relaxation) and a decrease of the intercept of the dynamic stiffness-tension line. These temporal changes in the isometric twitch and viscoelasticity properties appeared to parallel the development of hypertrophy since they were observed in senescent but not middle-age or young adult rats. LV hypertrophy of a similar extent as that occurring in senescence was induced in middle -aged rats by aortic banding. Measurement of aortic input impedance demonstrated that the acute load induced by the banding was small being manifested only by a 15% increase in characteristic impedance. The same spectrum of changes in both isometric twitch and viscoelasticity coefficients as those seen with senescence were demonstrated in these muscles from hearts hypertrophied by banding. However, the magnitude of the changes in the mechanical properties were still significantly less than those observed in senescence. While the findings of this study do not prove a causal relationship between hypertrophy and the functional alterations with senescence, they are suggestive that at least a portion of the functional alterations are due to the underlying hypertrophy. The reasons for the quantitative differences between the banding and senescent hypertrophy are open to speculation.

Significance to Biomedical Research and the Program of the

Institute: The results are significant to biomedical research because they encourage further studies to elucidate the mechanism for the age difference in relaxation and stiffness. If this can be dtermined it will extend our knowledge regarding the contraction and relaxation process in heart per se. The results are significant to the program of the Institute in that two of the most distinctive features of aged hearts of many species, including man, are hypertrophy and slowing of relaxation. This is of potential clinical significance during times of stress, at high heart rates, when diminished relaxation could interfere with ventricular filling and lead to enhanced dyspnea and functional impairment. It appears that the diminished filling and slowed relaxation may be in part due to the hypertrophy per se.

Proposed Course: To determine whether physical conditioning will alter either the relative hypertrophy, and/or increased stiffness and/or slowed relaxation in the senescent heart.

Publications:

Lakatta, E. G.: Alterations in the cardiovascular system that occur in advanced age. Fed. Proc. 38: 163-167, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00031-01 CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Length-Dependence of Excitation-Contraction Coupling in Cardiac Muscle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	H. A. Spurgeon	Physiologist	CPB, NIA

Other: None

COOPERATING UNITS (if any)

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Recent evidence suggests that the mechanism underlying Starling's law of the heart involves a length-dependence of excitation-contraction process, or in other words, at shorter muscle lengths, contractile activation is less than at longer lengths. In isolated mammalian cardiac muscle, the precise mechanism for the length-dependence of contractile activation are sought.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To measure the effect of muscle length on the kinetics of the force staircase in isolated cardiac muscle.

Methods: Papillary muscles are studied in a standard myograph. (1) Quiescent muscles were stimulated to contract regularly and the force production which increased with each beat (for staircase) until a new steady level of force development was achieved was measured. (2) In muscles contracting regularly in a steady state, the Ca^{++} in the bathing fluid was abruptly changed and the ensuing force staircase measured. The staircases at different muscle lengths are recorded and the kinetics at each length are measured via a computer program.

Major Findings: (1) When stimulated from quiescence at 24 min^{-1} in a bathing fluid $[Ca^{++}]$ of 2.5 mM, 16 of 23 muscles exhibited biexponential increases in force production at both lengths. The coefficients of the exponential function at L_{max} were 1.5 to 2 times greater than their counterparts at the shorter length, and this length difference was highly significant. When the force staircases were normalized to the peak developed force attained at each length, the number of beats to attain 25, 50, 75 and 98% of peak force at 0.85 L_{max} was approximately twice that required at L_{max}.

(2) At a given length the force staircase (a) exhibited a dependency on the number of beats rather than on stimulation frequency over a range of 12-60 min^{-1}, (b) was accelerated by increasing the bathing fluid $[Ca^{++}]$ from 1.0 to 5.0 mM, (c) was accelerated in the presence of isoproterenol, and (d) was retarded in the presence of d-l verapamil. Over the entire range of bathing fluid $[Ca^{++}]$ and at all stimulation frequencies 24 min^{-1} and above, more beats were required to complete a given level of the normalized staircase at 0.85 L_{max} than at L_{max}. There was no length difference in the presence of verapamil. These data suggest that transsarcolemmal Ca^{++} influx is an important determinant of the kinetics of the force staircase, and the length-dependence of the latter indicates that muscle length is an important determinant of transsarcolemmal Ca^{++} influx.

(3) This conclusion was strengthened by the results of studies in which the $[Ca^{++}]$ of the bathing fluid was abruptly increased from 1.0 to 5.0 mM with the muscle beating in the steady state. The resulting increase in force production (Ca^{++} staircase) was described by a monoexponential function with a greater coefficient at L_{max} than 0.85 L_{max}; when normalized to the peak force difference in the two $[Ca^{++}]$ at each length, a given level of the staircase was achieved in significantly fewer beats at L_{max}

than at the shorter length. This data has been presented at the fall meeting of the American Physiological Society, October 1978 (Lakatta and Spurgeon, The Physiologist 21: 69, 1978). The manuscript describing these data has also been accepted for publication (vide infra).

Significance to Biomedical Research and the Program of the Institute: Starling's law of the heart, or increased muscle performance at longer lengths has according to "classical theory" in heart muscle physiology, been attributed to the change in sarcomere geometry that accompanies the change in muscle length. The present results cannot be explained on the basis of sarcomere geometry and indicates that an additional mechanism, length-dependence of contractile activation, accounts at least in part for the length-dependence of force production. The data provide a mechanism which in part, explains the length-dependence of excitation-contraction coupling in cardiac muscle with intact sarcolemmae.

Proposed Course: To measure the kinetics of staircase of the cardiac intracellular action potential at different muscle lengths in order to further elucidate the apparent length modulation of Ca^{++} flux.

Publications:

Lakatta, E. G., and Spurgeon, H. A.: Force staircase kinetics in mammalian cardiac muscle: modulation by muscle length. J. Physiol. (Lond.) (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00032-01 CPB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Contractile and Biochemical Responses to Catecholamines in the Hyperthyroid Heart

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	C. Filburn	Staff Fellow	LMA, NIA
	E. Beard	Chemist	CPB, NIA
Other:	None		

COOPERATING UNITS (if any)

T. Guarnieri, Department of Medicine, Johns Hopkins Medical Institutions

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The apparent increase in adrenergic activity observed in the organism in the hyperthyroid state in the presence of a normal level of circulating catecholamines may result from enhanced responsiveness to basal or threshold concentrations of catecholamines. This may result from an increase in the number of β-receptors demonstrated recently. The purpose of the present study was to measure the cAMP-dependent protein kinase activation and contractile response subsequent to threshold levels of β-stimulation with isoproterenol in the hyperthyroid and euthyroid heart. These studies were also performed at maximal doses of isoproterenol. In addition, since the protein kinase mediated phosphorylation of sarcoplasmic reticulum and subsequent enhancement of Ca^{++} transport appears to be integral to the contractile response, we also measured stimulation by cAMP-dependent kinase of Ca^{++} accumulation in sarcoplasmic reticulum enriched microsomal preparations from euthyroid and hyperthyroid hearts.

Project Description:

Objectives: The objectives of these studies are to characterize (1) the near threshold and maximal response to contractile performance and biochemical mediators of contractile performance in hyperthyroid and euthyroid hearts to β-adrenergic stimulation, and (2) the stimulation of Ca^{++} accumulation velocity by cAMP and protein kinase in microsomal preparations isolated from hyperthyroid and euthyroid hearts.

Methods: (1) Perfused interventricular septal preparations from hyperthyroid and euthyroid rats were employed in this study. Contractile performance was measured under (a) basal conditions, (b) during low levels of β-adrenergic stimulation (10^{-9} isoproterenol), and (c) during high levels of stimulation (10^{-6} isoproterenol). Under each condition the septum was quick frozen and analyzed for the level of cAMP-dependent protein kinase activation. (2) In other studies, sarcoplasmic reticulum enriched microsomal preparations were isolated from euthyroid and hyperthyroid hearts. Basal and stimulated (cAMP-dependent kinase) Ca^{++} accumulation velocity were measured.

Major Findings: The contractile response measured as maximal rate of force development (dF/dt) to a near threshold concentration of isoproterenol (10^{-9}M) was enhanced in perfused interventricular septa from hyperthyroid (128±4% control) compared to euthyroid rats (105±2%, $P<.01$). This enhanced contractile response was accompanied by a significant activation of cAMP-dependent protein kinase (protein kinase activation ratio increased from .159±.008 to .218±.019, $P<.005$, while no significant changes from baseline occurred in euthyroid septa .152±.007 to .179±.012, NS). No difference between hyperthyroid and euthyroid hearts was observed in the contractile response to $1X10^{-4}$ dibutyryl cAMP (126.5±2.5% and 122.0±9.2% in hyperthyroid and euthyroid, respectively). The magnitude of the response to dibutyryl cAMP was comparable to that observed in the hyperthyroid group with 10^{-9} isoproterenol. These results suggest that the mechanism for enhanced protein kinase activation and contractile response to low concentrations of isoproterenol in the hyperthyroid heart is at or proximal to cAMP generation. The maximum contractile response to isoproterenol ($5X10^{-7}$M), however, was decreased in hyperthyroid myocardium (192±13%) compared to euthyroid (291±37%, $P<.05$). Both protein kinase activation ratio (.356±0.017 and .344±0.013) and the maximal contractile response to Ca^{++} (335±15% and 340±12% control in hyperthyroid and euthyroid, respectively) were similar, suggesting that the mechanism of the diminished maximum response was distal to protein kinase activation but not a function of an altered Ca^{++}-troponin interaction. The diminished maximum dF/dt response in the hyperthyroid hearts was accompanied by significantly less shortening of the contraction duration which was 85.6±2.1% control in hyperthyroid vs 66±2.8% control in euthyroid, $P<.001$. Although the basal rate of Ca^{++} accumulation was greater in microsomes isolated from hyperthyroid than from euthyroid hearts there was a significantly diminished increase in Ca^{++} accumulation in response to exogenous cAMP and protein kinase in hyperthyroid compared to euthyroid hearts. This reduction may explain the diminished effect of isoproterenol on the shortening of contraction duration in hyperthyroid compared to the euthyroid myocardium, and may explain, at least in part, the diminished maximum response to isoproterenol in dF/dt. Part of these data have been presented at the Scientific Sessions of the American Heart Association, October 1978 (Guarnieri et al., Circulation 58: II-20, 1978) and a manuscript has been submitted for publication.

Significance to Biomedical Research and the Program of the Institute: The enhanced threshold contractile response, which we have demonstrated for the first time, provides a mechanism to explain in part the apparent hyperadrenergic state in hyperthyroidism. The results also provide a logical explanation why treatment of hyperthyroidism with β-adrenergic blocking agents has been successful.

Proposed Course: To determine whether the enhanced threshold contractile response to β-adrenergic stimulation in the hyperthyroid heart is mediated by a greater increase in slow inward Ca^{++} current compared to the euthyroid heart. These studies will be performed in an electromechanical isolated muscle preparation.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00033-01 CPB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Ambulatory Electrocardiography in a Healthy Elderly Population

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Fleg	Staff Cardiologist	CPB, NIA
Other:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA

COOPERATING UNITS (if any)

H. L. Kennedy, Div. of Cardiology, USPHS Hospital, Baltimore, MD

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.2	OTHER: 0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although anti-arrhythmic medications and cardiac pacemakers are used most frequently in the elderly, relatively little attention has been focused on characterizing the patterns of the heart beat in normal elderly subjects over an extended period. In the current project, 100 healthy men and women from the Baltimore Longitudinal Study (BLS) over the age of 60 will undergo ambulatory electrocardiography for a 24-hour period, during part of their regular 2 1/2 day visit to the Gerontology Research Center. A small 2-channel Holter recorder will be employed.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The prevalence of supraventricular and ventricular ectopy of all types, conduction disturbances, disorders of impulse formation, repolarization abnormalities and characterization of diurnal variations will be obtained over a 24-hour period via a 2-channel Holter monitor.

Methods: One hundred men and women from the Baltimore Longitudinal Study (BLS) over the age of 60 will be studied. Subjects with treated hypertension or a BP more than 160/95, diabetes requiring drug or dietary therapy, overt or suspected heart disease, or chronic obstructive lung disease will be excluded. Heart disease will be ruled out by history and physical exam, ECG, maximal treadmill exercise test and chest x-ray. Patients with an FEV_1 less than 60 percent of their predicted value will not be studied.

A small 2-channel Holter recorder weighing about 1 lb. will be attached to the patient after informed consent has been obtained. The subject will wear the device for a 24-hour period, while he undergoes the standard battery of tests for participants in the BLS. He will be asked to keep a diary of activities and symptoms (particularly chest pain, dizziness, palpitations or shortness of breath) on a standard form which is routinely used for that purpose. At the end of 24 hours, the recorder will be detached and the tape send to Drs. Harold Kennedy and Dennis Caralis at the USPHS Hospital for analysis.

The tapes will be analyzed by a high speed scanning system with manual validation. Hourly quantitation of ventricular and supraventricular premature beats (VPB's and SVPB's) will be made as well as changes in heart rate and ST segments. This data will be correlated with the extensive information available on these subjects (activity, smoking pattern, weight, nutritional history, etc.). The prognostic significance of the Holter monitor findings should be readily assessed by clinical followups of these BLS participants over a 5-year period.

Major Findings: Supraventricular and ventricular ectopy occur in the majority of a healthy active geriatric population. High degree AV conduction disturbances, profound sinus bradycardia and ischemic repolarization patterns are rare in normal elderly subjects.

Significance to Biomedical Research and the Program of the Institute: Because such a large percentage of healthy elderly subjects demonstrates ectopic beats, the significance of such findings in patients with cardiac symptoms is diminished unless the symptoms and dysrhythmias occur concomitantly. Profound sinus bradycardia and high degree atrioventricular block, however, should be considered distinctly abnormal.

Proposed Course: Ambulatory electrocardiographic studies should be extended to include younger and middle aged participants in the BLS. Holter monitoring should be combined with ambulatory automated blood pressure recording in normals, hypertensives, and patients with cardiac and cerebrovascular disease.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AG-00093-07-CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Cellular Basis of Regulation of the Humoral Immune Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. A. Nordin	Research Chemist	CPB NIA

COOPERATING UNITS (if any)
None

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 1.7	PROFESSIONAL: 0.8	OTHER: 0.9

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The regulatory mechanisms of lymphoid cells are being investigated in vitro using a T-cell dependent as well as a T-cell independent antigen. The cellular requirements and functions involved in the in vitro immune response are being established for normal adult mice. These are then being compared to those in aged mice of the same strains. The possibility that regulatory mechanisms observed in young adult mice are amplified in old mice resulting in immunosenescence is being investigated.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The goal of this project is to characterize the cells regulating the immune response by cellular elements in both young and aged mice. Efforts to determine the origin and mechanism of action of these cells are of prime interest.

Methods Employed:

(1) The *in vitro* culture techniques and the assay for plaque-forming cells are routine methods.

(2) The carbonyl-iron treatment of spleen cells is accomplished by adding 25 mg of sterile carbonyl iron to 100 x 10^6 normal spleen cells. After a 30 minute incubation at 37°C in a 5% CO_2 environment, the iron and cells with ingested iron are removed by magnetic attraction. This process is repeated and the spleen cells free of iron-ingesting cells are used as a source of T and B lymphocytes.

(3) Peritoneal exudate cells are collected from unstimulated mice and used as a source of accessory cells. These cells are either used directly or an adherent layer prepared from them before other cell types are added. Supernatants of peritoneal cells collected 24 hours after culture initiation are used in some instances instead of the peritoneal cells.

(4) DAGG-Ficoll is prepared by modifying Ficoll by introducing carbonyl methyl amino-ethyl groups to which is added the tri-peptide glycine-glycl-alanyl with the terminal alanine substituted with a single dinitrophenol haptenic group. The preparation used here contains 48 moles of hapten per mole of Ficoll.

Major Findings: The *in vitro* immune response to the T-independent antigen, DAGG-Ficoll, requires the presence of macrophage-type cells or a factor(s) derived from them. Spleen cells of nu/nu mice respond to DAGG-Ficoll when cultured *in vitro* but the magnitude of the response can be increased 5 fold if splenic T-cells derived from nu/+ mice are added to the cultures. This helper effect does not require that the T-cells be prepared from previously immunized mice and in this respect differs from the helper effect seen with T-dependent antigens. Spleen cells of nu/nu mice depleted of macrophages by treatment with carbonyl-iron do not respond to DAGG-Ficoll even with the addition of nu/+ T-cells. If macrophages or a factor(s) derived from them, are added to identical cultures, the responsiveness of the nu/nu spleen cells is restored.

Spleen cells from 24 month old C57BL/6 female mice were examined to determine the cellular deficiencies responsible for the low *in vitro* response to DAGG-Ficoll. With respect to the function of macrophages, of 21 mice examined 10 animals had a response equivalent to that observed in young mice. The remaining 11 animals had various levels of activity with only 2 animals showing no activity. With respect to the function of T-cell activity in aging mice, spleen cells from 24 month old animals were cultured with normal syngeneic T-cells in the presence of macrophages derived from PC of young mice. Of 18 old mice examined 5 showed levels of activity equivalent to that observed in young mice. The remaining old mice showed various levels of activity and of these, 9 mice showed no or minimal increase in activity.

In vivo and in vitro administration of thymopoeitin to 24 month old C57BL/6 female mice has not influenced their in vitro response to DAGG-Ficoll. Various doses and schedules were employed including implantation of osmatic pumps to deliver the drug continuously for 7 days. Spleen cells from these animals when cultured in the presence of macrophages or factor derived from young animals did not respond significantly different from control cultures.

Significance to Biomedical Research and the Program of the Institute: The goal of this research program is to examine the cellular populations that are regulating the humoral immune response. The mechanisms by which the regulation takes place would be of significance not only to the field of immunology but would have relevance to cell biology. It is also significant to the area of immunosenescence. The decline in immunological responsiveness with age is well established but the reasons are not at all understood. The role of regulatory mechanisms in explaining the phenomena of immunosenescence may be of considerable significance.

Proposed Course: Initial attempts at purification of the macrophage derived factor(s) indicate that the active component can be separated from the great bulk of serum protein present in the tissue culture medium. This will be pursued in an effort to prepare sufficient factor for determining the mechanism(s) involved in the cellular interactions leading to antibody formation and to begin selected chemical studies on the nature of the factor. Several established macrophage cell lines will be examined to determine if they produce the factor(s). An active cell line would be invaluable in our attempts to prepare significant quantities of the factor(s).

The studies with aging mice will be continued to determine if the low response to DAGG-Ficoll observed in approximately one-half the animals, despite the addition of T-cells and macrophages from young animals, is due to a reduced B-cell population or an increased suppressor cell activity.

Publications:

Nordin, A. A. and Adler, W. H.: The Effect of Aging on In Vitro Cellular Interactions. In Siskind, G. W., Litwin, S. D. and Weksler, M. E. (Eds.): Developmental Immunobiology, New York, Grune & Stratton, 1979, pp. 215-229.

Nordin, A. A.: The in vitro immune response to T-independent antigen. I. The effect of macrophages and 2-mercaptoethanol. Eur. J. Immunol. 8: 776-781, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AG-00094-06-CPB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Characterization of Immune System of Aging Mice with Immunodeficiency

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. A. Nordin	Research Chemist	CPB NIA
Other:	A. J. Russo	Staff Fellow	CPB NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.1	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to characterize the in vitro cell-mediated immunity in young and aging mice of various genetic strains. The relationship between the immunodeficiency in aging and the regulation, in the form of suppression of the immune system, is investigated. The topics of present interest are: (1) the regulatory mechanisms of the in vitro development of cytotoxic lymphocytes by suppressor cells, (2) the characterization of suppressor cells, and (3) the characterization of the immunodeficiency in aging and its relationship with suppressor cells.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The goal of this project is to characterize the immune system in young and aging mice of various genetic strains. The relationship between the immunological disorders and the regulation, in the form of suppression of the immune system, is investigated.

Methods Employed: (1) A modification of the spleen cell culture system of Mishell and Dutton is used. Spleen cells from individual mice are cultured with mitomycin-C treated or irradiated allogeneic spleen cells, F_1 spleen cells or heterologous erythrocytes at 37°C for various days. The double chamber culture system is also used.

(2) Cytotoxicity assay - ^{51}Cr labelled EL-4 or P-815 cells are mixed with cultured spleen cells and incubated for various times. After incubation, cold PBS is added, the tubes centrifuged and the radioactivity of the supernatant counted.

(3) Plaque-forming cell assay-routine technique used to detect IgM and IgG antibody-producing cells.

(4) A radioactive (^{51}Cr) micro tube leukocyte adherence inhibition assay was used. Mononuclear cells from murine spleen were separated using Ficoll-Hypaque, labelled with ^{51}Cr, and 5×10^4 spleen cells are incubated in microculture plates, with flat bottom wells (37°, 5% CO_2), for 1 hr with specific or control tumor membrane extracts. The C.P.M. of labelled non-adherent cells were used as a parameter of adherence.

Major Findings: Specific and reproducible leukocyte adherence inhibition was measured using a modified radioactive micro assay in the MCA-38 colonadenocarcinoma, P-815 mastocytoma, and L1210 lymphoma murine tumor systems.

Although thymus derived lymphocytes (non-adherent) by themselves did not undergo adherence inhibition, they were necessary for the specific immune response. L.A.I. was ablated by treating spleen cells with anti thy 1.2 serum and C′ and could be re-established with the addition of thymocytes. Splenic adherent cells were not responsive in the L.A.I. assay. Immunoglobulin forming cells were not required for specific adherence inhibition since treatment of spleen cells with anti Ig serum and C′ had no effect on the L.A.I. assay. Serum from mice with progressively growing tumors could also cause normal cells to become less adherent in the presence of tumor extract.

A comparison between the radio-L.A.I. and cytotoxicity assays established that both assays reproducible measure immune response to specific tumor. Normal spleen cells and spleen cells from tumor bearing mice express natural (non-specific) cytotoxicity against both K562 and P-815 labelled cells. This natural killing is suppressed in the spleen population from the tumor bearing animal as a function of tumor size.

The *in vitro* development of cytotoxic lymphocytes (CL) is accompanied by the expression of suppressor cells. This suppression can be shown to be the result of soluble factor(s) found in the supernates of cultures 2 days after initiation of mixed lymphocyte cultures (MLC). The factor is titratable and incorporation of 25 μl of an active supernate in 200 μl MLC significantly reduces the expression of CL. The factor(s) is not directly cytotoxic since cell recovery is not significantly reduced and the response of spleen cells to PHA, Con A and LPS is not affected by the addition of a suppressive MLC supernate.

Significance to Biomedical Research and the Program of the Institute: This proposal offers two main significant contributions: (1) the regulatory mechnaism of cell-mediated immunity in young and aged mice, and (2) the mechanism of the immunosenescence of cell-mediated immunity.

Proposed Course: The effect of serum from tumor bearing mice on the L.A.I. assay will be investigated further. Attempts will be made to partially purify the serum component responsible for the suppressive effects and establish the mechanism involved. Also, several cell lines are now under investigation as potential target cells in the L.A.I. assay. A consistent target cell line would be of considerable help in the attempts to further investigate the significance of the cellular activity being assessed by L.A.I. assay.

The radio-L.A.I. and microcytotoxicity assays will be used to measure the immune response of normal and tumor bearing aging mice in a effort to establish 1) the response to specific tumor in aging mice and 2) the level of suppression of immune response, as measured by natural (non-specific) and induced cytotoxicity in aging mice. Further study of the mechanism of suppression of natural killing by spleen cells from tumor bearing mice will be pursued.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00095-06-CPB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Role of Cell Membrane Structures on Cellular Recognition

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. H. Adler	Medical Officer, PHS	CPB NIA
Other:	J. E. Nagel	Clinical Associate, PHS	CPB NIA
	R. K. Saxena	Visiting Fellow	CPB NIA
	Q. B. Saxena	Visiting Fellow	CPB NIA
	R. Yanagihara	Clinical Associate, PHS	CPB NIA EOD 7/1/79

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	2.3	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project will define the functional capability of lymphocyte subpopulations in aging mice. Assays for functional ability and techniques for cellular manipulation will allow for correlation of type with particular function. The use of a cell sorting machine will facilitate separation of subpopulations of cells in lymphoid tissue. The analysis of cellular function in aging animals will delineate the basis of the age associated decline in immune function.

Project Description:

Objectives: To correlate certain immunologic functions with morphologically identifiable populations of immunologically active cells. The functional criteria and results will be compared to arrive at correlations in order to develop methods for diagnosing and describing immune deficiency and assigning certain predictive projections of immune function. The tests of immune functions will include responses to mitogen and antigen in in vitro culture conditions, responses to antigens and oncogenic stimuli in vivo, and the development of immunogically competent cells in in vitro environments. These studies will give a better understanding of age-related immunodeficiency.

Methods Employed: The basis of most functional assays will be in vitro culture systems. There will be both short term for the investigation of mitogen and antigen responsiveness and for the generation of antibody-forming cells, and longer time for the generation of cytotoxic lymphocytes and antibody-forming cell colonies. In vivo methods will primarily be cell transfer studies and transplantation studies with syngeneic tumor cells. The cells will be from various lymphoid organs and from varying aged donors. The cells will be treated by physical separation methods and with specific antisera to eliminate certain populations, or to quantitate various cellular population representation. The use of a cell sorter with appropriate antisera will allow separation of distinct subpopulations.

Major Findings: T-cell memory in a cytotoxic system in which mice were immunized with an allogeneic tumor cell suspension was analyzed over a 2 year period post immunization. The ability of the spleen cells from the immunized mice to generate a secondary response on in vitro challenge was used as the assay for memory. The findings showed that memory decays to low levels at one year post initial immunization. It is easily detectable at 6 months post immunization. Therefore, T-cell memory is relatively short lived.

Levels of natural killer cell activity in mice can be shown to be strain dependent and also able to be augmented by antibody directed against cell surface antigens on the effector cells. Using these studies it can be shown that effector cells do not carry theta antigen or immunoglobulin but do carry histocompatibility antigen and antigens in the Ia group. This assay will detect a function in a non T or B-cell population in mouse spleen and allow a measure of age related change in that population.

Experiments using thymopoietin administration to old mice have shown that there is no effect on the spleen cells ability to respond to mitogens. The old mice which have been treated respond as poorly as the non-treated mice. Other assays of immune function may show an effect of thymic hormone thymic hormone therapy and this is being investigated.

The immunologic response of mice of varying ages to Pneumococcal antigen is being investigated. The antibody response appears to be the same over all age groups tested. The effects of a pneumococcal infection, however, in the immunized mice are much more lethal in the older age groups.

Studies on bacterial killing by mouse spleen cells also shows a marked defect in the ability of cells from old mice. These studies will be expanded to delineate host-parasite interaction in old animals.

Significance to Biomedical Research and the Program of the Institute: We are gaining a better definition and appreciation of the term immunodeficiency. Since a relative immunodeficiency is seen in aging, it is important to develop better diagnostic criteria, so that possible remedial measures can be undertaken. The studies on the resistance of old animals to infection will help to outline theraputic regimens for dealing with a major problem in elderly humans.

Proposed Course: To continue to outline the connection between form and function and to expand our technical ability to measure functions. To develop better diagnostic criteria and tests to describe immune capacity.

Publications:

Nordin, A. A. and Adler, W. H.: The Effect of Aging on *In Vitro* Cellular Interactions. In Siskind, G. W., Litwin, S. D. and Weksler, M. E. (Eds): *Developmental Immunobiology*, New York, Grune & Stratton, 1979, pp. 215-229.

Adler, W. H. and Chrest, F. J.: The Mitogen Response Assay as a Measure of The Immune Deficiency of Aging Mice. In Siskind, G. W., Litwin, S. D., and Weksler, M. E. (Eds.): *Developmental Immunobiology*, New York, Grune & Stratton, 1979, pp. 233-248.

Nariuchi, H. and Adler, W. H.: Dissociation between proliferation and antibody formation by old mouse spleen cells in response to LPS stimulation. *Cell*. *Immunol*. 45: 295-302, 1979.

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Adler, W. H., Jones, K. H. and Brock, M. A.: Ageing and Immune Fucntion. Chap. 13 in Behnke, J. A., Finch, C. E. and Moment, G. B. (Eds.): *The Biology of Aging*. New York, Plenum Press, 1978, pp. 221-234.

Jones, K. H. and Adler, W. H.: The decay of memory of cell-mediated cytotoxicity after primary sensitization *in vitro* against a tumor allograft. *Immunol*. *Commun*. ed. 6, 7: 645-659, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AG-00096-06-CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Low Temperature Effects on Cells of Aging Individuals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. A. Brock	Research Biologist	CPB NIA
Other:	W. H. Adler	Medical Officer, PHS	CPB NIA

COOPERATING UNITS (if any)
None

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to characterize possible age-related differences in the in vitro responses of murine lymphohemopoietic cells to mitogens and their differential susceptibility to freezing damage. Because there are optimal cooling velocities for the cryopreservation of each cell type, T- and B-lymphocytes may be recovered with slight impairment of function using an optimal cooling rate. Changes in cellular structure and/or function with age then may be distinguishable with selective freezing injury to one or both subpopulations. Structures considered the sites of freezing damage are the plasma membrane and cellular membrane systems.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To characterize the functional capacity and structure of pre- and post-mitotic cell types from aging individuals, specifically the possible age-related differences in (1) the in vitro responses of human and murine lymphohemopoietic cells to various agents and (2) the differential susceptibility of murine and human immunocompetent cells to freezing damage assessed by their functional recovery after thawing.

Methods Employed: Cell suspensions were prepared from spleens of C57BL/6 mice. The percentage of viable lymphocytes in the unfrozen suspensions and in those that had been cryopreserved was determined using the stains, fluorescein diacetate and ethidium bromide, to identify viable and non-viable cells, respectively. Suspensions of the lymphocytes were adjusted to 15 or 30 x 10^6 cells/ml in media (RPMI-1640) containing 10% DMSO and 10% fetal calf serum. They were cooled in glass bottles at rates ranging from -0.25° to -10.0° C/min. to the low limit of -50°C using the microprocessor-controlled rate controller developed in this laboratory. After storage in the vapor phase of liquid N_2 (-196° C), the cell suspensions were rapidly thawed and the DMSO diluted before culture in vitro. The incorporation of ^{3}H-thymidine into DNA was used to assess the mitotic activity of the cells after in vitro activation of T-lymphocytes by the mitogens, phytohemagglutinin (PHA) and Concanavalin A (Con A), and B-lymphocytes by lipopolysaccharide (LPS).

Major Findings: The viability and functional recovery of cryopreserved lymphocytes were compared to their unfrozen controls in splenic cell suspensions from young (4-6 mo old) and 15 mo old mice. At the optimal cooling velocities which ranged from -0.5° to -5.0° C/min, 60-100% of the unfrozen cells from young mice were recovered, whereas only 40-55% of the unfrozen cells were viable in 4 of the 10 cryopreserved cell suspensions from 15 mo old mice. Recoveries ranged from 62-78% in the other 6 samples from the older mice. At the sub-and supraoptimal cooling velocities, -0.25° and -10.0° C/min, recovery of lymphocytes from young mice fell to 47-69%. Despite the differences in the percentages of cells recovered, there was no selective injury to either the T-cell or B-cell populations from young mice at cooling rates ranging from -0.25° to -10.0° C/min. This was shown by the nearly unchanged ratios of T-cell to B-cell function, that is, the ratios of the incorporation of ^{3}H-thymidine in response to stimulation by T-cell and B-cell mitogens. The ratios were unchanged even at the cooling rate of -10° C/min where the incorporation of ^{3}H-thymidine fell to less than 50% of the unfrozen cell suspensions. In contrast, in cell suspensions from 15 mo old mice cooled at -1.0°, -2.5° and -5.0° C/min, the T-cell population response to mitogens was selectively depressed in all except two suspensions cryopreserved at -5.0° C/min. B-lymphocytes in response to stimulation by LPS incorporated from 30-100% of the ^{3}H-thymidine of their unfrozen controls. This large variation is also in contrast to B-cells from young mice which incorporated 90-100% of the ^{3}H-thymidine of their unfrozen controls at cooling velocities ranging from -0.25° to -5.0° C/min. This selective impairment of function of lymphocytes from older mice occurred despite the fact that their plasma membranes were intact as judged by the fluorescein stain for viability.

Significance to Biomedical Research and the Program of the Institute: The reported decline in the functional capacity of lymphocytes with age may be intrinsic and/or extrinsic. These possibilities can be tested by modifying components in an *in vitro* system which tests functional capacity and by assessing the effects of freeze-thaw damage on lymphocytic biomembrane systems. Controlled rate cooling is a new technique that may be used to preserve lymphocytes for further study at the cellular level or for therapeutic use.

Proposed Course: Changes with age in lymphocytic resistance to the stress of cryopreservation will be continued using controlled rate freezing techniques. The characterization of possible age-related changes in mammalian lymphoid cells will include further studies on the enhancement of splenic lymphocytic responses to mitogens as these may be related to cellular biomembrane systems.

Publications:

Adler, W. H., Jones, K. H. and Brock, M. A.: Ageing and Immune Function. Chap. 13 in Behnke, J. A., Finch, C. E. and Moment, G. B. (Eds.): *The Biology of Aging*. New York, Plenum Press, 1978, pp. 221-234.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AG-00104-03-CPB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Clinical Immune Survey of the Longitudinal Project Participants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. H. Adler	Medical Officer, PHS	CPB NIA
Other:	A. A. Nordin	Research Chemist	CPB NIA
	J. E. Nagel	Medical Officer, PHS	CPB NIA
	R. K. Saxena	Visiting Fellow	CPB NIA
	Q. B. Saxena	Visiting Fellow	CPB NIA
	R. Yanagihara	Clinical Associate, PHS	CPB NIA EOD 7/1/79

COOPERATING UNITS (if any)

None

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 3.95	PROFESSIONAL: 2.05	OTHER: 1.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The immune function of an aging human population will be evaluated to determine the age associated changes and the possible effects on the incidence and types of disease. The individuals demonstrating decreased immune function will be evaluated closely in this regard. The eventual goals will be to determine which age related diseases are associated with a decrease in immune function and therefore can be modulated by augmenting the function. The accurate assessment of function of the human population will be a major goal with the development of newer assays and the refinement of existing assays.

Project Description:

Objectives: The purpose of this project is to assess different immune functions and to correlate these results in order to gain an overall picture of the individuals' immunologic ability. Since there are many assays that can be performed, each is presented individually with the understanding that through the correlational analysis of the data it may be possible to arrive at the most useful assays to employ in measuring human immune function.

The three broad areas of the program are data collection, research and service. The data collection and research aspects are dealt with specifically in terms of investigation of serum antibody and lymphocyte responses. The service aspect is a consultative function in setting up assays that use immunological methods, such as radioimmune assays for hormones, or fluorescent antibody staining in histopathology.

The specific assays of human immune function which are used in this study are as follows.

1. The measurement of serum immunoglobulin levels by radial-diffusion.

2. The measurement of specific antibody to immunogens, such as diphtheria or tetanus toxins, and pathogens, such as pneumococci, influenza, E. coli, etc.

3. The measurement of peripheral blood granulocyte function.

4. The identification and quantitation of subpopulations of lymphoid cells in the peripheral blood.

5. One assay of immune lymphocyte function is the response of lymphocytes to mitogens for T cells (PHA, Con A), B cells (PWM, SA), and antigens (Diphth Tox, Tet Tox, Candida).

6. The measurement of the T cell-helper effect will be incorporated into the assay systems used to evaluate immune function. The necessity of thymus-derived lymphocytes (T cells) to interact with bone marrow-derived lymphocytes (B cells) to produce antibody to most antigens has been well established. Since there is an impressive amount of evidence that T cell function declines with age, it is not unreasonable to suspect that the decline of antibody synthesis with age is a result of a lack or malfunction of this helper cell activity associated with T cells.

7. Another assay will be the determination of the number of lymphocytes in peripheral blood which can differentiate to antibody-forming cells. There is evidence to indicate that immunoglobulin synthesis in aging mice and humans becomes restricted and often converts to a monoclonal nature. However, it is not yet appreciated if such a restriction in any way affects the integrity of the immune system.

8. The measurement of Epstein-Barr virus (EBV) infection will be accomplished by determining if cells from older humans will form established EBV infected lymphoblast cell lines in tissue culture. We will also determine the presence of anti-EBV antibody in the serum as evidence of exposure.

Methods Employed:

1. Lymphoid cell preparations:
   a. Human peripheral blood lymphocytes (PBL) will be isolated from freshly drawn heparinized blood of the participants of various age groups in the program. The blood is diluted with an equal volume of tissue culture medium carefully layered over Ficoll-Hypaque and the tube centrifuged. The lymphocytes are retained at the interface and are easily collected. The cells are thoroughly washed to remove any Ficoll-Hypaque and resuspended in a tissue culture medium. These cells will be identified and cultured in standard systems. Granulocyte preparations are obtained by dextran sedimentation of the red cells in the pellet from the F-H separation with subsequent collection of granulocytes from the dextran mixture.

2. Tissue culture and assay:
   a. The human PBL are mixed at various concentrations with a fixed number of murine B cells and cultured for four days. The antigen used to stimulate the cultures will be sheep erythrocytes.

   b. The detection of antibody-producing cells will be by the plaque assay which allows a quantitative estimate of the number of cells actually synthesizing antibody. Therefore, we can titrate the helper cell effect and establish the level of the effect as opposed to an "all or none" type of information.

   c. The detection of cytotoxic T lymphocyte activity will be by chromium isotope labelling of the target tumor cells and then incubation with the lymphocytes. The measure of isotope release by the target cells will be the measure of the cytotoxicity induced by the lymphocytes.

3. Assays of immunoglobulin synthesizing cells will be accomplished by plating pokeweed-stimulated blood lymphocyte cultures with sheep red cells that have been coated with Protein A. The development of plaques will be accomplished using specific antisera and a complement source.

4. EBV titers will be accomplished by standard methods using lymphoblast cell lines, serum titration and a fluorescent anti-immunoglobulin antisera.

5. A cell sorter will be utilized to study specific subsets of lymphocytic populations.

Major Findings: To date 373 individuals have been examined using the various assays. Thirty one individuals have been examined a second time and 77 of 163 (47%) women have been examined, all for a first time.

The results are at present analyzed in an age-cross sectional fashion except for the peripheral blood cell count studies which are longitudinal studies over 5 cycles and 10 years. The findings are:

1. No decrease with age of peripheral blood lymphocyte immunoglobulin synthesis induced with pokeweed mitogen.

2. An age related decrease in T lymphocyte proliferative responses to mitogens. There is no age related decrease in B lymphocyte proliferative responses.

3. No age related change in serum immunoprotein levels.

4. No age related change in peripheral blood lymphocyte natural killer cell activity.

5. No change in the levels of peripheral blood white cells, lymphocytes, granulocytes, monocytes, T-cell rosetting cells and surface immunoglobulin positive B-cells.

6. The T-cell helper assay has not been applied to the analysis of immune function because of technical variability and difficulty which may preclude its use on a large population.

7. The levels of anti-pneumococcal antibody in the longitudinal population increases with age.

8. A micro-system for analysis of peripheral blood granulocyte bacterial killing has replaced the measure of metabolic activity as a standard function test. The metabolic studies were too variable to provide quantitative data on a population although they are useful for a qualitative judgment on cell function. The new assay may provide a better assessment of granulocyte function.

9. Lymphoblast lines have been established on longitudinal subjects with a 15% success rate. Analysis of the lines and the individuals who donated cells is proceeding.

Significance to Biomedical Research and the Program of the Institute: There is no doubt that these studies, along with the many other pieces of information available on this population, will yield valuable data to show the presence or absence of a correlation of aging diseases and disorders with a deficiency of immune function. At present, there is no information on this subject. If we find that an age-associated immunodeficiency does lead to specific diseases, we could, hopefully, avoid the disorders by augmenting immune function. It may also be possible to alter our immunogens so as to make them more effective in the aging host.

Proposed Course: The number of subjects studied has increased and within the next two years immune function data will be available for all participants in the longitudinal program. This year we have also started to obtain data on the female participants. It is planned over the next few years that the data from the immune function assays will be compared to other data on these individuals. The types of questions to be asked will be; what are the effects on immune function of certain drugs, diseases, gender, and diet? What is the effect on disease incidence and severity on decreased immune function? In all our population studies there are always individuals who demonstrate levels of immune activity outside the normal range. These individuals will be chosen for more careful scrutiny in terms of the above questions. Lastly, we will begin to restudy individuals from previous years to determine the stability of immune function over a one to two year period, so that, we can determine the interval necessary for reassessment of function.

Publications:

Adler, W. H., Jones, K. H. and Brock, M. A.: Ageing and Immune Function. Chap. 13 in Behnke, J. A., Finch, C. E. and Moment, G. B. (Eds.): The Biology of Aging. New York, Plenum Press, 1978, pp. 221-234.

NIA ANNUAL REPORT
OCTOBER 1, 1978 THROUGH SEPTEMBER 30, 1979
OFFICE OF PLANNING AND EXTRAMURAL AFFAIRS

The Office of Planning and Extramural Affairs (OPEA) was established in June 1979, under the direction of Dr. Don C. Gibson, Associate Director. With the creation of this new office, the functions of planning and evaluation, grant referral and review, legislative analysis, program and policy analysis, international affairs, and program coordination are integrated into one organizational unit for the first time. These functions of OPEA are grouped into three organizational components.

Planning, Evaluation, and Legislative Analysis

OPEA staff is responsible for developing NIA plans and planning procedures, planning for and monitoring evaluations of Institute programs, and providing information and analysis on legislative activities. Responsibilities also include the coordination of interagency and international activities of the NIA.

In FY 1979, staff members were responsible for developing NIA's first set of guidance materials on internal planning and managing the first complete cycle of program review activities. In the area of evaluation, NIA's evaluation plan was prepared and approved and implementation plans for approved projects to be initiated in FY 1980 are nearing completion. Evaluation staff is also leading an effort to develop comprehensive performance indicators for NIA research programs.

Legislative activities have included the monitoring of a wide variety of legislative issues and proposals, including research and training in geriatrics, mental health of the elderly, long-term care, retirement of commercial airline pilots, cancer and aging, and the upcoming World Assembly on Aging. Staff has also prepared testimony, position papers, and special initiative documents on such subjects as geriatric training and research, health promotion and disease prevention, specialized centers of research, middle-aged women, hospice, and the role of research in health care cost-containment.

Referral, Review, and Training

OPEA staff is responsible for providing initial scientific merit review on program projects, training grants, clinical investigator awards, and geriatric medicine academic awards through the Aging Review Committee (ARC) and the *Ad Hoc* Geriatric Medicine Academic Award Committees. It also coordinates NIA policies, procedures, and programs related to training, assigns applications by program area within NIA, and participates in the National Advisory Council review of applications. The staff handles a diversified group of applications in the basic and clinical aspects of the biological, behavioral, and social sciences. Applicants for program project grants or institutional training grants are usually site-visited by a team composed of members of the ARC, the ARC Executive Secretary, extramural scientists, and a grants management representative. Twenty-two site-visits were made in FY 1979. The ARC reviewed 29 grant applications requesting over $39 million in FY 1979, and recommended approval on 21 proposals for a total cost of over $9.8 million. Work is underway to refine NIA guidelines and procedures for grant referral.

Program Analysis

OPEA staff is responsible for the organization, retrieval, analysis and interpretation of NIA scientific and administrative data. Based upon these data, Institute staff makes decisions concerning all aspects of NIA activities and development and prepares responses to Executive, Congressional, and public inquiries about research supported by NIA.

To this end, Program Analysis staff is developing an NIA Management Information System (MIS). The first segment of the MIS, which handles basic reporting needs of NIA, including grants referral and management, animal models census and projection, budgetary data, and extramural program information, was implemented in 1979. The addition of a graphics capability to provide automated graphic displays of program data will complete this segment of the system. The second phase of the MIS, already under development, will permit the capture and analysis of data specific to NIA contracts.

Concurrently, a scientific data base is being developed. This will provide precise scientific categorization of all extramural projects, as well as systematic summarization of extramural research in progress, and will enable NIA staff to identify and examine the content of other Institutes' age-related projects.

Program Analysis staff recently published an index of current research grants and contracts administered by the NIA and will soon publish summaries of extramural research and training grants. Guidelines and procedures have also been developed for grant referral, assignment, and follow-up, and are documented in an NIA Policy and Procedure Statement.

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